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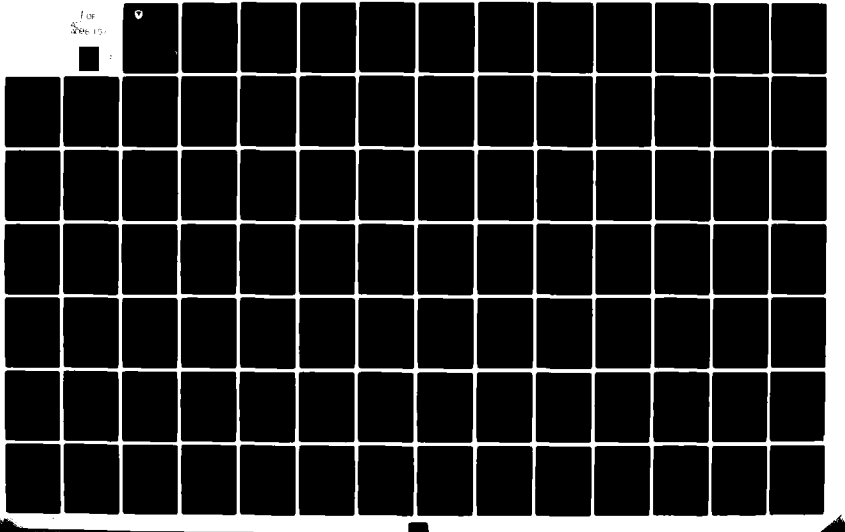

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HANDBOOK

FOR EVALUATING ECOLOGICAL EFFECTS OF POLLUTION AT DARCOM INSTALLATIONS

VOLUME 5

AQUATIC SURVEYS

MAY 1980

U.S. ARMY DUGWAY PROVING GROUND
Dugway, Utah 84022

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Volume 5 of seven volumes dealing with evaluating ecological effects of pollution at DARCOM installations

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aquatic ecosystems	periphyton
information systems	aquatic macroinvertebrates
military activities	fish

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

This handbook provides the DARCOM commander with a tool whereby he can respond quickly to a potential or actual pollution incident with a decisive program to evaluate the ecological effects of the pollution. To implement the procedures as set forth in the handbook, the commander will enlist the help of an environmental team composed of DARCOM scientists (or other suitable personnel) and individuals with limited ecological training (paraecologists) who will do much of the manual labor. With a given volume, the paraecologist can perform the required functions. (cont)

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Item 20 (cont)

The handbook covers the following areas in seven volumes of which this is Volume 5: (1) basic questions that need answering, (2) conducting the preliminary investigation of the problem, (3) determining the specific effects of a pollutant (the first three volumes are essentially library efforts), (4) terrestrial sampling, (5) aquatic sampling, (6) unexpected declines in animal populations and (7) handling data.

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CONCEPT

Pollution is a biological problem because its primary affect is on living organisms. Historically, water pollution has been monitored using chemical, physical, or biological techniques. Although the three techniques have been regarded as separate and competitive, they complement each other and must be considered simultaneously in a pollution ecology survey (PES).

Chemical monitoring measures the concentrations and kinds of material present in the water and sediments. Physical monitoring measures currents, morphometry, sunlight, and characteristics of the sediments in the receiving waters. Biological monitoring measures the kinds and numbers of organisms and their response to physical and chemical conditions in the environment. However, because certain indicator[1] organisms selectively bioaccumulate, biomagnify, and show synergistic effects of exposure to environmental stresses, they have the potential for revealing an environmental stress before it is detected by chemical and physical monitoring methods.

The enforcement of discharge standards for domestic and industrial wastewaters was granted by Congress to the U.S. Environmental Protection Agency (EPA) in Sections 301 and 302 of the Federal Water Pollution Control Act (FWPCA) of 1972[2]. In addition to chemical and physical monitoring, compliance includes biological monitoring. Each state is required to develop water quality standards[3] which include a characterization of the biological effects. The EPA enforces the state water quality standards and effluent limitations by issuing discharge permits [National Pollutant Discharge Elimination System (NPDES) Permits] which can include requirements for biological monitoring[4]. Section 502 of the FWPCA[5] defines biological monitoring as "the determination of the effect [of an effluent, discharge, or compound] on aquatic life, including accumulation of pollutants in tissues ... by techniques and procedures including sampling of organisms representative of appropriate levels of the food chain ...". The U.S. Army's commitment to

[1]For a complete discussion of the criteria for an acceptable biological indicator, refer to Concept, Vol. 4 (p iv).

[2]33 U.S. Code (U.S.C.) Sections (Sec.) 301 and 302, 1972.

[3]40 Code of Federal Regulations (C.F.R.), Part 120, as amended, 6 June, 1978.

[4]40 C.F.R., Part 125, 16 May 1973.

[5]33 U.S.C. Sec. 502, 1972.

monitoring and pollution control is defined by Executive Orders 11514[1] and 11752[2].

This volume provides guidance and procedures that are necessary to accomplish aquatic monitoring with emphasis on biological methods.

[1]Executive Order (Ex. Ord.) No. 11514, 5 March 1970, 35 Federal Regulation (F.R.) 4247.

[2]Ex. Ord. No. 11752, 17 December 1973, 38 F.R. 34793.

USE OF THIS VOLUME

This volume describes the procedures for conducting aquatic pollution ecology surveys. It is divided into six chapters: (1) the considerations for selecting appropriate sampling sites, and the characterization of (2) water, (3) sediment, (4) periphyton, (5) aquatic macroinvertebrates, and (6) fishes at each sampling site.

The team leader will determine the general site for collection, procedures required, quantity and size of the samples, frequency of sampling, and other special requirements. The paraecologist will have the primary responsibility to learn and apply many aquatic sampling techniques.

Each procedure in Chapters 2 through 6 includes a brief purpose, introductory notes, a description of sampling techniques, sources of error, equipment checklist, and a list of useful references.

The methods selected may not prove to be the best for every situation. If they are not adequate and new methods are identified, request the information be sent to:

Commander
U.S. Army Dugway Proving Ground
ATTN: STEDP-MT-L
Dugway, UT 84022

for possible inclusion in a revision of this volume.

FOREWORD

This volume was revised by David A. Gauthier and Dr. Carlos F.A. Pinkham.

This volume was prepared by Dr. Edward S. Bender[1], MAJ Jay Abercrombie, and SP5 Robert J. Donald[2], U.S. Army Chemical Systems Laboratory, Aberdeen Proving Ground, MD 21010.

In addition, the following people have contributed information used in Volume 5:

Christina M. Wheeler, U.S. Army Dugway Proving Ground, UT 84022

James H. Wheeler, U.S. Army Dugway Proving Ground, UT 84022

Henry Eichorn, U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD 21010

NOTE

The pronoun "he" is used in this volume as an impersonal pronoun which encompasses both he and she and has no intent of personal reference or connection.

[1]Present address: Compliance Branch, Office of Water Enforcement, U.S. Environmental Protection Agency, Washington, DC 20460.

[2]Present address: Eastern Regional Health Center, Baltimore, MD 21237.

NOTES

CHAPTER 1 - PRELIMINARY CONSIDERATIONS

CONTENTS

a. SITUATIONS REQUIRING MONITORING

(1) Point Source Discharge

A point source discharge enters the receiving waters from a pipe, storm sewer, or treatment pond at a specific point. Often monitoring of the point source discharge may be included as a requirement for a National Pollutant Discharge Elimination System (NPDES) Permit. This monitoring provides the plant manager with data that he can use to detect system upsets, violations of water quality standards, and changes in the manufacturing process or treatment efficiency.

(2) Non-Point Source Discharge

A non-point source discharge enters the receiving waters from groundwater, runoff, or fallout. Numerous activities such as operation of waste disposal sites, maintenance areas, motor pools, test areas, training facilities, and laboratories (refer to Section C, Volume 2) may emit non-point source pollutants. When these pollutants enter the water, their impact must be evaluated before a method of containment or abatement can be established.

(3) Accidental Spills and Fish Kills

Occasionally, a commander must respond to incidents such as spills of chemicals or unexpected declines in populations of aquatic organisms.

Volume 6 will be consulted in such cases. After an incident has occurred, a survey is conducted to determine the impacts on the terrestrial and aquatic communities. The results of this survey are used to determine the presence of residual chemicals and their potential health hazards and to assess the effects of the incident. The survey must be conducted as soon as possible so that the impact of the incident can be assessed and any changes caused by the cleanup can be monitored.

b. ESTABLISHING REFERENCE AND EXPERIMENTAL AREAS

Data from a body of water in an experimental area (an area where pollution is suspected) are meaningless without some reference to what the area was like before the pollution. The reference area is selected and studied to establish estimates of the natural variations of the chemical and physical characteristics of the body of water and the complement of organisms which occur in the absence of pollution.

The first step in the selection of proper reference areas is to establish a baseline of the ecosystems in the vicinity of the experimental area so that a number of candidate sites can be selected for further study and appropriate biological indicators can be chosen. The literature survey outlined in Volumes 2 and 3 will determine whether the necessary baseline information is available. If not, we recommend using the procedures outlined in the Study of Ecological Classification and Inventory Manual[1], which uses field observations to establish a baseline. Once the baseline has been established the procedures in Volume 5 will be used to conduct the PES.

If a point source discharge enters a flowing body of water (stream or river), reference conditions should be determined from an area that is upstream of the influence of the discharge. Transects which dissect the parts of the stream of interest will be established. A transect contains two or more sample sites a known distance apart. The location of the transect will be permanently marked with T-posts, flags, etc., and its location noted on the survey map (Appendix A). This assures that the same sites will be sampled during subsequent visits. If the discharge enters a standing body of water (lake or pond), reference conditions will be determined from an area that is not influenced by the discharge, preferably within the same body of water. In a lake or impoundment, sample sites should be placed on transects radiating out from the discharge point as lines or as a series of semi-circles. Data collected from radial sampling in the experimental area would be used to

[1]U.S. Department of the Navy, Naval Facilities Engineering Command, Alexandria, VA 22332, Study of Ecological Classification and Inventory Manual, M.M. Goodwin, Oct 1977.

determine the effects of the pollutant(s) at various distances from the discharge, while data in the reference area would be used to sort out naturally occurring differences due to changes in depths, substrates, etc.

In general, aquatic surveys for non-point sources will be based on factors similar to those in a survey of point source emissions. However, the extent of the non-point source must be defined by preliminary sampling for the pollutant. Unlike the point source discharge, the amount of pollutant emitted from a non-point source will vary with run-off, leaching, and degradation of the pollutant. Topography, drainage, and vegetative cover will affect the locations where the pollutant enters the stream, in which case, selected techniques from Volume 4 may be required. More specific locations for sampling areas usually can be determined from a topographic map. The environmental scientists will select areas which appear to have similar drainage, topography, and vegetational characteristics for the reference (upstream) and experimental (downstream) areas. In lakes or impoundments comparisons can be made between open water areas or between areas near the shoreline.

The selection of sampling areas from a map is then verified by field reconnaissance. The environmental scientists will examine the physical habitats present at each area: (1) to determine the sampling techniques to be used, (2) to eliminate extraneous or arbitrary differences between areas (such as the influence of man-made structures or unusual flow characteristics), and (3) to assure that an adequate number of reference areas is available to compare with each type of habitat in the experimental areas. Field reconnaissance should include photographs and measurements of physical boundaries of the habitat.

c. ESTABLISHING THE NUMBER OF SAMPLE SITES WITHIN EACH REFERENCE AND EXPERIMENTAL AREA

The outstanding physical characteristic of streams in the unidirectional, fluctuating current. The speed of the current often dictates the principal habitat of the stream. Riffles occur in areas of high current and pools in areas of low current.

The habitats of greatest invertebrate animal production in streams are riffles. They may contain darters, midges, stoneflies, mayflies, hellgrammites, caddisflies, snails, crayfish, and many other aquatic animals. They are also the feeding and spawning ground for trout, smallmouth bass, and other fish. Dissolved oxygen content is high and primary production (plant material) is generally limited to attached algae and planktonic diatoms.

From the riffles to the pools there is a drastic change in the biota. Lake forms tend to replace stream forms. In pools, primary production is generally higher than in riffles if siltation and

organic pollution are not high enough to cause low dissolved oxygen conditions (3 milligrams per liter or less). Pools are often inhabited by invertebrates such as amphipods, pond snails, sludgeworms, leeches, and burrowing mayflies and vertebrates such as frogs, catfish, creek chubs, suckers, and minnows.

Although there are major differences between riffles and pools, minor differences may occur within each habitat type throughout the stream or from one side of the stream to the other. Therefore, sampling should include as many sites in each area as possible. The team leader must balance available resources and scientific requirements to determine the intensity and scope of the sampling.

d. ESTABLISHING THE SAMPLING TECHNIQUES

The nature of the pollutant, its environmental fate, and timing will determine which medium (surface water, water column, or sediments) in the receiving water is sampled and thereby which sampling techniques to use. For example, materials of low solubility are suspended in fast flowing water or settle to the bottom in calm water. Materials which are suspended have their primary impacts on plankton and fish and have secondary impacts on the periphyton and invertebrates that live in or on the sediments. Deposited materials have primary impacts on the benthos (sediment-dwelling organisms) and on the spawning and development of fish populations.

The rates and schedules of release of the pollutant will influence the frequency of sampling. For example, if the wastewater is released intermittently, it is more likely to have an adverse impact on the aquatic community than the same wastewater discharged continuously. The plants and animals in the community are more likely to adapt to the continuous discharge.

In every case, all samples must be collected at one sample site before proceeding to the next sample site. The order of sampling at each sample site should be water, biological, and sediment. Whenever flow is evident or suspected, always sample the downstream sample site first and proceed upstream (up current) to the subsequent sample sites. Failure to follow these procedures may result in false samples or contamination of samples at one sample site with sediment and organisms from another sample site.

e. RECORD KEEPING

Detailed records of the investigation are essential. Appendix A contains a discussion of the techniques that will help ensure proper documentation of the study. Follow the techniques described explicitly.

An accurate description of a sample site is critical: (1) to interpret the results of the survey, (2) to locate the site for future sampling, and (3) to document the conditions existing during sampling at that location.

Fill out a general information sheet (Figure 1-1) on each transect. Some entries will be provided by the environmental scientists. Others will come from subsequent chapters. Obtain the "Drainage Area" by identifying the boundary of the drainage system on a U.S. Geological Survey topographical map of the appropriate scale and measuring the area within the boundary with a planimeter (described in Chapter 2, Volume 4). The boundary of a drainage system is the ridgeline between all tributaries leading into the drainage system under study and all tributaries leading into other drainage systems. Check the appropriate entry for "Dominant Substrate" and "Dominant Flow". "Summer Temperature" and "Winter Temperature" refer to the average surface temperature during the respective periods. Width is measured bank to bank with a cloth or metal tape at several points at each transect. Record the distance in meters.

Fill in the sample site diagram with a sketch of the locations and identification numbers of the sample sites along the transect. Include enough surrounding features to convey the exact location of the transect to others. Include a north arrow and scale.

f. SHIPMENT OF SAMPLES

If the capability to identify or analyze samples does not exist at your installation, it will be necessary to ship the samples elsewhere. Appendix B provides the necessary instructions.

NAME OF WATER BODY _____ TRANSECT _____

LOCATION _____ DATE SURVEYED _____
(Incl township & range, if possible)

COUNTY _____ STATE _____

Drainage Area _____ km^2 Pool/Riffle Ratio _____

Dominant Substrate: _____ Gravel _____ Course Sand _____ Fine Sand _____ Silty Clay _____

Dominant Flow: _____ None _____ Temporary _____ Permanent

Summer Temperature _____ °C Winter Temperature _____ °C

Banks/Shores _____
(Describe)

_____

_____

Special Features _____
(Describe)

_____

_____

Dominant Nearby Terrestrial Vegetation _____
(Describe)

_____

_____

Dominant Aquatic Vegetation _____
(Describe)

_____

_____

Sample Site Diagram Width _____ m
(on a separate sheet)

_____

Figure 1-1. General Information Sheet for Each Transect

CHAPTER 2 - WATER SAMPLING

CONTENTS

a. INTRODUCTION

Changes in the biological composition of aquatic communities are usually the result of physical and chemical changes. Organisms better adapted to the new conditions become dominant. Organisms unable to adapt decline and may disappear completely. For example, rooted aquatic plants become established along the edges of streams with a low flow gradient. If flow and turbulence increase, the abrasive force of sediments will remove the vegetation. If the stream velocity remains constant and the nutrient load is increased, the plants will thrive and may completely choke the stream's flow.

b. MEASUREMENT OF WATER CHEMISTRY

Water is never absolutely pure in nature. As a result of leaching, runoff, precipitation, and the life cycles of organisms, natural waters contain different amounts of salts, nutrients (such as nitrogen, carbon, phosphorus, and sulfur), and organic compounds. The chemical properties of natural water will influence the behavior of pollutants that are discharged into the water and the kind of organisms that are present. Therefore, an aquatic survey must include measurements of the chemical characteristics of the water at each sample site. This includes measurements that are made on-site and those made in the laboratory. The paraecologist will perform the on-site field measurements; for laboratory measurements, the paraecologist will collect the samples and, depending on the complexity of the analysis, may analyze them.

(1) Procedure

(a) Field Measurements

Dissolved oxygen (DO) must be measured in the field, generally by membrane electrodes or the Winkler (iodometric) method.

Membrane electrodes are very convenient but require a special piece of equipment (available from Yellow Springs Instrument Company,[1] which also furnishes instructions for its use). The oxygen membrane electrode is adequate for most field measurements, but it must be calibrated to compensate for water temperature and conductivity.

The Winkler method is the most accurate and reliable procedure for DO analysis.[2] The Hach Chemical Company[3] has developed a kit for analyzing DO in the field. The kit includes instructions, a special collection bottle, prepackaged chemicals for the azide modification of the Winkler method of DO analysis, and narrow-mouthed, glass-stoppered, biochemical oxygen demand (BOD) bottles of 300 milliliter (mℓ) capacity.

To obtain samples for DO analysis:

Collect samples with a Kemmerer[R4], Van Dorn sampler, or the collection bottle in the Hach kit.

Follow instructions supplied with sampler or kit.

Drain the sample from the sampler or collection bottle through a tube extending to the bottom of a 300-ml BOD bottle.

Fill the BOD bottle to overflowing (continue overflow for approximately 10 seconds). Avoid turbulence and formation of bubbles while filling the bottle.

---

[1]Yellow Springs Instrument Company, Yellow Springs, OH 45387.

[2]Standard Methods for the Examination of Water and Wastewater, 14th ed. American Public Health Association, Washington, DC, pp 440-454, 1976.

[3]Hach Chemical Company, Ames, IA 50010.

[4]Wildco, 301 Cass St., Saginaw, MI 48602

Once the BOD bottle is filled to overflowing, chemically fix the sample (by the Winkler method), and insert the stopper, allowing excess water to flow over the rim. See Table 2-1 for storage requirements.

Analyze the sample for DO and record the results on a water-sample data sheet (Figure 2-1). Also refer to Appendix A.

Record the temperature to the nearest tenth of a degree Celsius (C). Use instructions on p 2-11.

Another important chemical parameter measured in the field is pH (the concentration of hydrogen ions in a solution). A neutral pH is 7.0; pH values above 7.0 are alkaline; pH values below 7.0 are acidic. Measure the pH as soon as the sample is taken, because inaccuracies can be caused by temperature change, photosynthesis, and bacterial respiration. There are many battery-operated systems with an accuracy of ±0.1 pH unit. Each system is supplied with instructions for calibration and operation.

(b) Laboratory Measurements

The procedures for water quality analyses are described in Standard Methods for the Examination of Water and Wastewater.[1] The specific analyses performed will be determined by the team leader, based on the nature of the pollutant and the purpose of the survey. Record data on a water-sample data sheet (Figure 2-1).

Water samples can be collected manually as grab samples or automatically with a sequential or composite sampling device.[2] Collect the samples in clean containers, i.e., containers washed with 1 Normal nitric acid solution and rinsed three times with distilled water. The sample containers should be glass; each lid should have a Teflon^{R} lid liner. Plastic containers are acceptable for metal analyses. Label each sample as described in Appendix A. Include the sample site, date, time and the types of analyses to be made from the sample. When the container is filled, place it in an ice chest. Procedures recommended for the sampling and preservation of water samples for various analyses are included in Table 2-1.

---

[1] Standard Methods for the Examination of Water and Wastewater, 14th ed. American Public Health Association, Washington, DC, pp 440-454, 1976. The EPA may require use of Methods for Chemical Analysis of Water and Wastes.

[2] A sequential sampling device collects several samples automatically at pre-selected time intervals. A composite sampling device collects one sample consisting of one or more subsamples automatically taken at a pre-selected time interval.

Table 2-1. Recommendations for Sampling and Preservation of Samples for Chemical Analysis[a]

Measurement	Volume Required (ml)	Container[b]	Preservatives	Maximum Holding Time
Acidity	100	P, G	None	24 Hours
Alkalinity	100	P, G	Cool, 4°C	24 Hours
Arsenic(total)	100	P, G	HNO_3 to pH 2	6 Months
BOD	1000	P, G	Cool, 4°C	6 Hours[c]
Bromide	100	P, G	Cool, 4°C	24 Hours
COD	50	P, G	H_2SO_4 to pH<2	7 Days
Chloride	50	P, G	None	7 Days
Chlorine (total)	200	P, G	None	Det. on Site
Color	50	P, G	Cool, 4°C	24 Hours
Cyanides	500	P, G	Cool, 4°C NaOH to pH 12	24 Hours
Dissolved Oxygen				
Probe	300	G	None	Det. on Site
Winkler	300	G	Fix on Site	4 - 8 Hours
Fluoride	300	P, G	None	7 Days
Hardness	100	P, G	Cool, 4°C HNO_3 to pH<2	6 Months
Iodide	100	P, G	Cool, 4°C	24 Hours
MBAS[d]	250	P, G	Cool, 4°C	24 Hours

Table 2-1. Recommendations for Sampling and Preservation of Samples for Chemical Analysis[a] (cont'd)

Measurement	Volume Required (ml)	Container[b]	Preservatives	Maximum Holding Time
Metals				
Dissolved	200	P, G	Filter on Site HNO_3 to pH <2[e]	6 Months
Suspended	200	P, G	Filter on Site	6 Months
Total	100	P, G	HNO_3 to pH <2[e]	6 Months
Mercury				
Dissolved	100	P, G	Filter on Site HNO_3 to pH <2	38 Days (Glass) 13 Days (Hard Plastic)
Total	100	P, G	HNO_3 to pH <2	38 Days (Glass) 13 Days (Hard Plastic)
Nitrogen				
Ammonia	400	P, G	Cool, 4°C H_2SO_4 to pH <2	24 Hours
Kjeldahl	500	P, G	Cool, 4°C H_2SO_4 to pH <2	24 Hours
Nitrate	100	P, G	Cool, 4°C	24 Hours
Nitrite	50	P, G	Cool, 4°C	48 Hours
NTA[f]	50	P, G	Cool, 4°C	24 Hours
Oil & Grease	1000	G	Cool, 4°C H_2SO_4 or HCl to pH <2	24 Hours

Table 2-1. Recommendation for Sampling and Preservation of Samples for Chemical Analysis[a](cont'd.)

Measurement	Volume Required (ml)	Container	Preservation	Maximum Holding Time
Organic Carbon	25	P, G	Cool, 4°C H_2SO_4 or HCl to pH <2	24 Hours
Phenolics	500	G	Cool, 4°C H_3PO_4 to pH <4 1.0 g $CuSo_4/\ell$	24 Hours
Phosphorus				
Ortho-phosphate, Dissolved	50	P, G	Filter on Site Cool, 4°C	24 Hours
Hydrolyzable	50	P, G	Cool, 4°C H_2SO_4 to pH <2	24 Hours
Total	50	P, G	Cool, 4°C H_2SO_4 to pH <2	24 Hours
Total, Dissolved	50	P, G	Filter on Site Cool, 4°C H_2SO_4 to pH <2	24 Hours
Residue				
Filterable	100	P, G	Cool, 4°C	7 Days
Non-filterable	100	P, G	Cool, 4°C	7 Days
Total	100	P, G	Cool, 4°C	7 Days
Volatile	100	P, G	Cool, 4°C	7 Days
Settleable	1000	P, G	None	24 Hours
Selenium(total)	50	P, G	HNO_3 to pH <2	6 Months
Silica	50	P	Cool, 4°C	7 Days

Table 2-1. Recommendations for Sampling and Preservation of Samples for Chemical Analysis[a] (cont'd.)

Measurement	Volume Required (ml)	Container	Preservation	Maximum Holding Time
Specific Conductance	100	P, G	Cool, 4°C[g]	24 Hours
Sulfate	50	P, G	Cool, 4°C	7 Days
Sulfide	50	P, G	2 ml of 2N zinc acetate	24 Hours
Sulfite	50	P, G	None	Det. on Site[h]
Threshold Odor	200	G	Cool, 4°C	24 Hours
Turbidity	100	P, G	Cool, 4°C	7 Days

[a]The information in the table was taken from U.S. Environmental Protection Agency, Washington, DC 20460. Methods for Chemical Analysis of Water and Wastes, EPA-600-4-79-020, 1979. More specific instructions for preservation and sampling are found with each procedure as detailed in the above manual. This manual is available from Environmental Monitoring and Support Laboratory, 26 W. Clair St, Cincinnati, OH 45268.

[b]P = Plastic, G = Glass

[c]If samples cannot be returned to the laboratory in less than 6 hours and holding time exceeds this limit, the final reported data should indicate the actual holding time.

[d]Methylene blue active substances (surfactants)

[e]Where HNO_3 cannot be used because of shipping restrictions, the sample may be preserved initially be icing and shipped immediately to the laboratory. In the laboratory, the sample must be acidified to a pH <2 with HNO_3 (normally 3 ml 1:1 HNO_3/liter is sufficient). At the time of analysis, the sample container must be thoroughly rinsed with 1:1 HNO_3 and the washings added to the sample.

Footnotes Continued

[f]Nitrilotriacetic acid (indicator of several metals, particularly calcium, magnesium, zinc, copper, iron and manganese).

[g]If the sample is stabilized by cooling, it should be warmed to 25°C for reading, or temperature correction made and results reported at 25°C.

[h]Analyze immediately upon collection.

SAMPLE SITE_____

LOCATION_____
(Include township and range, if possible)

COUNTY_____ STATE_____

COLLECTION DATE_____ BY_____

WEATHER_____
(Describe)

Chemical Characteristics:

Depth (m)	DO (ppm)	Temperature (°C)	pH	Chemical/Chemical Property (Iron, lead, surfactant, etc)		
				Name	Name	Name

Physical Characteristics:

Turbidity_____m (Secchi disk transparency)

_____JTU[a]

Current Speed:

Length of Stream Measured _____m (A)

Time for Floating Object to Travel (A) _____sec (B)

$$\frac{(A)}{(B)} = \_\_\_\_\_ \frac{m}{sec}$$

[a]Jackson turbidity units (JTU) are comparable to formazin turbidity units (FTU) or nephelometric turbidity units (NTU)

Figure 2-1. Water-sample Data Sheet

(2) Shipment of Samples

Preserve and ship the samples according to the instructions from the receiving laboratory and the procedures in Appendix B.

(3) Sources of Error

Interference of sediment, metallic components, chlorides, and organic material with various chemical reactions (described in detail in Standard Methods for the Examination of Water and Wastewater[1]).

(4) Equipment Checklist Refer to Appendix C.

Sample bottles
Dissolved oxygen probe or apparatus for Winkler method
Kemmerer^{R} water sampler
300-ml BOD bottles
pH meter
Equipment and chemicals needed to collect samples for chemical analysis (Hach^{R} Chemical Kit or equivalent)

c. MEASUREMENT OF PHYSICAL CHARACTERISTICS OF THE BODY OF WATER

(1) Procedure

Important physical characteristics of the body of water at the sample site are turbidity, temperature, depth, and current speed. Record the results on a water-sample data sheet (Figure 2-1).

Turbidity can be measured with a Secchi disc, nephelometer, or turbidimeter. Instructions for the use of these instruments can be found in the manufacturer's instructions, Textbook of Limnology,[2] or Fundamentals of Limnology.[3]

[1]Standard Methods for the Examination of Water and Wastewater, 14th ed. American Public Health Association, Washington, DC, pp 440-454, 1976.

[2]Cole, G.A., Textbook of Limnology, C.V. Mosby Company, St. Louis, MO, 1975.

[3]Ruttner, R., Fundamentals of Limnology, University of Toronto Press, Buffalo, NY, 1969.

Flow is designated as none, temporary, or permanent. It is usually highest during rainy seasons and lowest in late summer. Navigable waters are often measured by stream gauges of the U.S. Army Corps of Engineers, U.S. Geological Survey, or state agencies (Refer to Appendix B, Volume 2). From these measurements, the expected low flow for several days over a period of years (e.g.; a 10-year, 7-day low flow), is often available for large streams and rivers.

Measure speed or flow rate in cm/sec. It can be obtained by noting the time for a floating object to travel a measured distance (in meters) along a stream. Flow rate is important because it determines which particles are dislodged from the substrate (Table 2-2). This affects the type of substrate that remains (Chapter 3). If the substrate is not the type expected from the measured flow, a fluctuating flow is indicated, which can be biologically significant.

Fill out the flow profile (Figure 2-2) for each transect. The flow profile depicts the current speed at even intervals along the transect (across the stream).

Measure water temperature in degrees Celsius. Water has a high specific heat capacity and therefore water temperatures fluctuate less than air temperatures. The density and oxygen-holding capacity of the water are inversely related to the water temperature. Water temperature controls the rates of chemical reactions and affects physiological processes. In lakes or impoundments layers of water may have different water temperatures, resulting in thermal stratification. Therefore, measure water temperature in a vertical profile (intervals to be determined by the team leader) whenever flow rates are low and depth exceeds 0.5 m.

(2) Equipment Checklist Refer to Appendix C.

Current meter
Thermometer
Graduated metal tape
Secchi disc, nephelometer, or turbidimeter
Meter stick
Temperature probe

d. SOURCES OF FURTHER INFORMATION

Cole, G.A., Textbook of Limnology, C.V. Mosby Company, St. Louis MO, 1975.

Hynes, H.B.N., The Ecology of Running Waters, University of Toronto Press, Buffalo, NY, 1970.

Table 2-2. Minimum Current Velocities Required to Dislodge Certain Soil Particles[a,b]

Velocity (cm/sec)	Soil Particle	Approximate Diameter (mm)	Dominant Characteristic
7.0	Fine Silts	0.0025 - 0.004	Low Cohesiveness
15.0	Clay	<0.002	High Cohesiveness
	Fine Sand	0.10 - 0.25	Angular
24.0	Course Sand	0.5 - 1.0	Angular
32.0	Pea Gravel	2.0 - 6.5	Spherical
	Small Gravel	6.5 - 12.5	Rounded
128.0	Gravel	30	Rounded
164.0	Stones	100 - 150	≥1 kg
256.0	Stones	≥900	≥60 kg

[a]Hynes, H.B.N., Ecology of Running Waters, Univ. of Toronto Press, Buffalo, NY, 1970.

[b]Guy, H.P., and V.W. Normal, Field Methods for Measurement of Fluvial Sediment, U.S. Geological Survey, Reston, VA 22092, Chapter C2, 1973.

NAME OF WATER BODY_____ TRANSECT_____

LOCATION_____ DATE_____
(Incl Township and Range, if possible)

COUNTY_____ STATE_____

Flow Profile (Composite of current speeds at all sample sites on the same transect)

Width of Stream_____m Depth of Profile_____m

Distance Between Sample Sites on Transects _____m

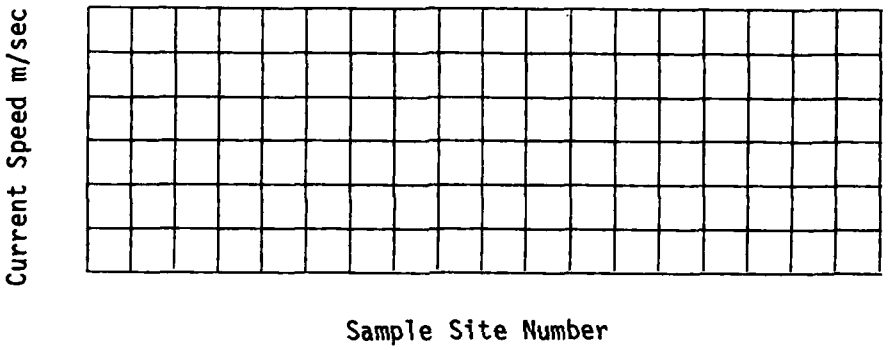

Figure 2-2. Flow Profile Data Sheet for Each Transect

Hynes, H.B.N., The Biology of Polluted Waters, University of Toronto Press, Buffalo, NY, 1970.

Ruttner, F., Fundamentals of Limnology, University of Toronto Press, Buffalo, NY, 1969.

Whitton, B.A. (ed), River Ecology, Studies in Ecology, Volume 2, University of California Press, Berkeley and Los Angeles, 1975.

CHAPTER 3 - SEDIMENT SAMPLING

CONTENTS

a. INTRODUCTION

Sediment is perhaps the single most important factor affecting the composition and diversity of an aquatic community at a particular site. Sediment chemistry and measurements of physical characteristics of the sediment are important for further understanding the ecosystem(s) in a given area. If clay is compacted into hardpan, it will support few organisms. Dense populations of invertebrates can be found in rubble when the current is swift and in finely divided silt and coarse debris in ponds or lakes where the current is slow or imperceptible.

b. MEASUREMENT OF SEDIMENT CHEMISTRY

Sediment samples are obtained for laboratory extraction and analysis of: (1) volatile materials, (2) organic compounds, and (3) metals and other inorganic compounds.

(1) Procedure

With core sampling devices, collect samples of stream and river sediments for organic and inorganic analyses. The core sampler maintains the layers of the sediment intact, so that specific layers or fractions of sediment samples can be examined and separated for chemical analysis. Perform all core sampling after collecting water samples and biological samples.

Based on the total number of core samples to be taken and the area to be sampled (established by the procedures in Chapter 1), transects will be established. In deep water (greater than wading depth) transects will be established by survey methods; in shallow water, transects will be located along a calibrated string from a fixed point.

If it becomes necessary to survey a site, consult a professional surveyor. Sample sites within a transect will be determined by random number selection (refer to Volume 7) for the core locations. Take cores from each transect starting at the downstream transect.

Collect three separate samples from each sample site for organic, volatile organic, and heavy metal analyses.

Before each sample is taken at a sample site, rinse all equipment with stream water. After each site is completed, wash the sampler in tap water, clean with a brush, and rinse with distilled water.

In shallow water obtain the sediment core with a Wildco[R1] hand-operated core sampler when you are collecting samples for all but inorganic analyses. For inorganic samples, push a plastic core tube liner from a Wildco[R2] gravity corer vertically into the sediment. Allow the core tube to fill with water, cap and withdraw. Remove the core from the liner by pushing with a core plunger rod from bottom to top.

In deep water use the appropriate core tube liner (borosilicate glass for metal or organics, plastic for inorganics) with the Wildco[R] gravity corer. Remove the core as above.

The necessity for separation of the core into subsamples will be determined by the team chemist. When separating the core, slice the core perpendicular to its long axis, using a metal spatula for organic samples and a plastic spatula for inorganic samples.

Place each core subsample in a separate, appropriate container (refer to Table 2-1) and use a waterproof marker to label the container with the sample site, subsample number, and date (refer to Appendix A). Remove excess water by decanting the supernatant from the jar after the sediments have settled.

If necessary, use Ekman grab samplers[3] to collect samples of detritus. Place the samples in separate jars labeled according to Appendix A. Return the jars to the laboratory for analysis.

Place all samples in a cooler and transfer to a refrigerator immediately upon returning to the laboratory.

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[1]Wildco Catalog No. 2420, 301 Cass St., Saginaw, MI 48602.

[2]Wildco Catalog No. 2410, 301 Cass St., Saginaw, MI 48602.

[3]Wildco Catalog No. 196, 301 Cass St., Saginaw, MI 48602.

(2) Sources of Error

Compression of the sample by the sampler piston

Disturbance of the fine bottom sediments as a result of lowering the sampler

Natural variations in sediment particle size

Natural variations in sediment water content

Absorption of organic material on the walls of the core tube (hand-operated core sampler) or core tube liners (gravity corer)

(3) Equipment Checklist Refer to Appendix C.

Clean[1] quart jars for organic and inorganic samples
Clean[1] quart jars with an organic solvent for volatile compounds. Each jar and solvent should be weighed, including the lid
Hand-operated core sampler with piston, or comparable device
A Wildco^R core sampler with a borosilicate glass liner (internal diameter 4.4 cm)
Core plunger rod
Plastic core tube liner with cap
Bottle brush
Tap water and distilled water in separate 5-gallon plastic carboys
Spatula
Brass Ekman grab sampler with handle or with messenger and rope
Brush to clean grab sampler

c. MEASUREMENT OF PHYSICAL CHARACTERISTICS OF SEDIMENT

Several physical characteristics are routinely measured in sediments: particle-size frequency distributions, percent silt and clay, and percent organic material.

(1) Procedure

The procedures described below are performed on a 25-cubic centimeter (cm^3) aliquot from a separate core sample. The aliquot may be obtained by slicing a 2-cm portion of the core, homogenizing the entire core, and removing about 25 cm^3 of sediment, depending upon the requirements of the PES. Place each aliquot in a glass jar and use a waterproof marker to label the jar with the sample site, aliquot number and date (refer to Appendix A).

[1]Clean jars by rinsing in the order indicated with 1 Normal nitric acid, hot soapy water, tap water, and glass distilled water.

(a) Particle-Size Frequency Distribution

The particle size classes will be determined by the mesh size openings of the sieves used. Usually three sieves are used: Number 10[1] (retains gravel), Number 40[1] (retains coarse sand), and Number 200[1] (retains fine sand).

Examine each sieve to be sure that it is dry and clean. With forceps, remove any sediment particles caught in the mesh.

Stack the sieves with the smallest number (largest mesh) on top, increasing to the largest number (smallest mesh) above the brass bottom pan.

Place stacked sieves on the shaker.

Transfer aliquot of sediment from the jar to a 50 ml glass beaker and oven dry at 105°C.

Weigh about 10 g of dry sediment on a dual beam balance. Pour the weighed sample into the top sieve pan. Cover and shake for 15 minutes.

Place total contents of each sieve pan on separate pieces of tared weighing paper. Use a brush to remove all particles from the pan to be certain all particles are included.

Weigh and record the sediment from each sieve on a substrate-sample data sheet (Figure 3-1).

Calculate the percent of each particle-size fraction by the appropriate equations in Figure 3-1.

(b) Percent Silt and Clay

The percent silt and clay (fine particles) is usually determined by wet sieving. Only one sieve pan (Number 200) and the brass bottom pan are used. Wash the sample with methyl alcohol to prevent cohesion of the fine particles and adhesion of the fine particles to larger particles. The percent of fine particles in the sediment correlates the concentrations of organic compounds present. More organics are found in sediments with a high silt-clay composition than in sediments with sand.

Place a small portion of the sediment sample on the Number 200 sieve with the brass botton pan beneath.

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[1]U.S. Standard Number designation.

NAME OF WATER BODY_____ SAMPLE SITE_____

LOCATION_____ DATE_____
(Incl Township and Range, if possible)

COUNTY_____ STATE_____

NOTES_____
(Presence of stones etc.)

_____

_____

Particle Size Frequency Distribution (Weights in mg)

Percent Gravel and Sand Fractions

Total Weight of Fraction _____ (A)

Weight of Gravel Fraction _____ (B) $\frac{(B)}{(A)}$ x 100: _____ = % Gravel

Weight of Coarse Sand Fraction _____(C) $\frac{(C)}{(A)}$ x 100: _____ = % Coarse Sand

Weight of Fine Sand Fraction _____(D) $\frac{(D)}{(A)}$ x 100: _____ = % Fine Sand

Percent Silt and Clay Fraction

Total Weight of Silt & Clay Sample ____(E)

Weight of Sample in Bottom Pan _____ (F) $\frac{(F)}{(E)}$ x 100: _____ = % Silt and Clay

Percent Organic Material in Silt and Clay Fraction

Weight of Crucible _____ (G)

Weight of Crucible and Dry Sediment _____ (H)

(H)-(G) = _____ Weight of Dry Sediment (I)

Weight of Crucible and Inorganic Ash _____ (J)

(H)-(J) = _____ Weight of Organic Ash (K)

(H)-(G)-(K) = _____ Weight of Inorganic Material

$\frac{(K)}{(I)}$ = _____% Organic Ash (Volatile Solids)

Figure 3-1. Substrate-sample Data Sheet

Wash and brush the sample gently, until all fine particles pass through the sieve.

Wash the contents of the sieve and bottom pan into separate tared beakers.

Dry the samples at 105°C. Cool in a dessicator and weigh each fraction.

Calculate percent silt and clay using the equation in Figure 3-1.

(c) Percent Organic Material (Volatile Solids) in Silt and Clay Fraction

Preheat muffle furnace to 600°C. Fire crucible for at least 12 hours, cool to room temperature in a dessicator and weigh.

Transfer dried silt and clay fraction (the fraction from the brass bottom pan) from the tared beaker to the tared crucible.

Weigh crucible and dry sediment.

Place crucible and sediment in the preheated muffle furnace and heat for 6 to 8 hours to incinerate organics. (If samples have clay in them, they may resemble pieces of pottery when dried.)

Cool in dessicator and weigh crucible and inorganic ash.

Calculate the percent organic material (volatile solids) using the equation in Figure 3-1.

(2) Sources of Error

Unequal loss of sediments from each fraction

Cohesion of sediment particles

(3) Equipment Checklist Refer to Appendix C.

Pint glass jars
Spatula
Homogenizer
U.S. Standard sieve pans of decreasing mesh sizes (i.e. Numbers 10, 40, and 200)
Brass bottom pan
Forceps
Rotap^{R} sieve shaker or equivalent
50 ml glass beaker
Dual beam balance
Drying oven
Analytical balance, sensitive to 0.1 mg or less
Weighing paper
Methyl alcohol
Camel-hair brush
Crucibles
Muffle furnace

d. SOURCES OF FURTHER INFORMATION

Anonymous, Soils Manual for Design of Asphalt Pavements Structures, Manual Series No. 10 (MS-10), Asphalt Institute, College Park, MD, Feb 1969.

U.S. Geological Survey, Reston, VA 22092. Techniques of Water-Resources Investigations of the United States Geological Survey, H.P. Guy, Book 3, Chapter C1, Fluvial Sediment Concepts, 1973.

U.S. Geological Survey, Reston, VA 22092. Field Methods for Measurement of Fluvial Sediment, H.P. Guy and V.W. Norman, Chapter C2, 1973.

NOTES

CHAPTER 4 - PERIPHYTON[1] SAMPLING

CONTENTS

a. INTRODUCTION

Periphyton is sampled because it is important in the aquatic ecosystem as food for fish and invertebrates. Periphyton forms a major photosynthetic community in many aquatic ecosystems; therefore, any impact on the periphyton will affect the entire ecosystem.

Diatoms, which can comprise up to 90 percent of the periphyton, are emphasized for the following reasons:

With some training most paraecologists can use the ornamentation of the silica frustule to identify diatoms to genus or species.

Much is known of the ecological requirements and environmental tolerances of many of the common species of diatoms, therefore, by

[1]Periphyton - The aquatic community (composed primarily of diatoms and other algae, bacteria, fungi, and protozoa) which grows on submerged objects. Other technical terms used here and in subsequent chapters are defined in Appendix D.

consulting ecological summaries of the abundant species (in references such as Environmental Requirements and Pollution Tolerances of Freshwater Diatoms[1]), inferences can be made about water quality.

Diatoms are found in almost all aquatic environments in large enough numbers for study.

Two approaches to sampling are recommended, depending upon the questions to be answered and the amount of time available for the survey:

A short survey of a few days in which existing substrates are sampled to determine the kinds and numbers of species present (species structure). A short survey quickly assesses the qualitative influences of pollution.

A long survey (7 to 30 days), using artificial substrate samplers to obtain quantitative estimates of standing crop (population size), biomass (weight of organisms present), net primary productivity (growth rate), and an index of organic enrichment (autotrophic index). A long survey provides quantitative data that can be used reliably to compare sites. For analysis of species structure, natural substrates should also be sampled during this long survey.

Although these surveys can be conducted independently, normally the long survey should follow the short survey to quantify and define effects encountered in the short survey. Together they provide the best estimate of species structure of periphyton at a sample site and provide a measure with which sample sites can be compared.

b. SHORT SURVEY

The length of the short survey depends on the number of sample sites and the accessibility of each. Normally it should last between 2 and 4 days. Artificial substrate samplers are used whenever existing substrates are inaccessible, such as in large rivers. However, at least 7 days are required to establish a community on artificial substrates.

(1) Procedure

At each sample site, use a knife to scrape samples from as many different substrates as can be found including rocks, submerged branches or roots, glass bottles, and beer cans. Also collect algal mats. Place scrapings from substrates into a glass jar half filled with a 4 percent

[1]Environmental Protection Agency, Washington, DC 20460. Environmental Requirements and Pollution Tolerances of Freshwater Diatoms, R. Lowe, 1974.

formalin preservative and use a waterproof marker to label the jar with sample site and date. These samples will be used for taxonomic and ash-free dry weight determinations (discussed later).

Use historical data or the standard method[1] test for hardness and salinity to determine whether the water is hard, soft, or brackish. The environmental scientist will estimate the extent of organic enrichment at the sample site. Enter these data on the short survey periphyton bench sheet (Figure 4-1).

(2) Sources of Error

The sources of error are not as significant for the short survey as they are for the long survey because quantitative sampling is not involved. Some researchers have found differences in community diversity due to shading. A species in an advantageous niche, such as a ridge in the bark of a submerged log, quickly multiplies to fill the niche. If only this section of the bark is scraped, a biased estimate of community structure will be obtained. The best way to keep this error minimal is to collect scrapings from as many substrates as possible and make a composite sample.

(3) Equipment Checklist Refer to Appendix C.

Sample jars
Knife
4 percent formalin (Appendix E)
Artificial substrates (optional)

c. LONG SURVEY

The length of the survey is based on the chlorophyll a accumulation rate. If chlorophyll a accumulation is graphed on the y-axis versus number of days in incubation on the x-axis, a sigmoid curve results. The most reproducible data occur when the community is most stable [at the point where the curve levels off (ending the period of exponential growth)]. The time required for a community to reach this point is usually 7 to 30 days, depending on season and nutrient levels. Stop sampling after that point is reached.

(1) Procedure

Fill slide rack of the periphyton artificial substrate sampler with 22 new, clean, standard glass microscope slides. The periphytometer

[1]Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, DC pp 99-120 (Salinity), 200-206 (Hardness), 1976.

SAMPLE SITE_____ DATE COUNTED_____

LOCATION_____
(Include township and range, if possible)

COUNTY_____ STATE_____

COLLECTION DATE_____ BY_____

COLLECTION METHOD_____ DAYS INCUBATED ON ARTIFICIAL SUBSTRATE_____

Site Characteristics

Hardness
(mg $CaCO_3$ eqv/ℓ)_____

Salinity
(mg NaCl/ℓ)_____

Organic Pollution (Check one)

No Organic Pollution _____

Some Organic Pollution_____

Polluted_____

First Transect

Total Number Counted _____ (A)
10% of (A) _____ (B)
Sum of Dominants Those > (B) _____ (C)
(A) - (C) _____ (D)

Value from Table 4-1 _____ (E)
$\frac{(E)}{(D)}$ = _____ (Total number of transects to be viewed)

Species	Number of Organisms

Figure 4-1. Periphyton Bench Sheet for Short Survey

should be constructed of plexiglass. The slide rack should be designed to hold the slides vertically, one cm apart. Attach styrofoam floats to the periphytometer so that the tops of the slides are submerged 1 to 3 cm below the surface of the water.

Commercial designs are available[1], or you can construct your own.[2]

Attach a flat styrofoam float to an anchor with stainless steel cable[3]. The length of the cable should be about 2 m more than the depth of the water at the sample site. Connect the sampler to the float with about 1 m of stainless steel cable. Simple supports may be used in small streams with stable flow rates.

Return every five days and remove four slides[4] from locations in the slide rack determined beforehand with a random numbers table (Volume 7). Using a razor blade, immediately scrape the periphyton from both sides and all edges of each slide into a separate 50 ml bottle (one slide per bottle). Discard the slides in an appropriate place.

To three of the four 50 ml bottles add 25 ml each of filtered sample site water.[5] Use a waterproof marker to label bottles with the sample site and date (refer to Appendix A) and then put bottles on ice in the dark. Use two samples for chlorophyll a determinations and one sample for ash-free dry weight determinations. Iced samples may be held 24 hours before processing. If delays of up to 30 days are necessary, freeze the samples at -20°C.

Preserve the periphyton in the remaining 50 ml bottle with 25 ml of four percent formalin. Label as above. This sample will be used for taxonomic determinations.

If further analysis of species structure is required, sample as many substrates (branches, rocks, macrophytes, etc.) as possible in the vicinity of the sample site.

---

[1]Design Alliance Corp., Columbus, OH 43205.

[2]Thomas, W.A.T. (ed.), Indicators of Environmental Quality, Plenum Publishing Corp., New York, NY 10011, 1975.

[3]Stainless steel is used to discourage vandalism and theft.

[4]If more than 25 days will be required, two samplers at each sample site will yield sufficient slides.

[5]Algal cells can rupture if they are placed in water of different conductivity or frozen. Sample site water is filtered through a Number 12 Watman^{R} paper or equivalent filter placed in a funnel.

(2) Sources of Error

Most of the errors for the long survey result in incomplete data:

Grazing by invertebrates (caddisflies, etc.). This is minor for the first week or two of incubation. It becomes more significant as the standing crop of periphyton increases, which results in an increase in the number of grazing insect larvae.

Scouring (In swift-flowing, turbid streams the suspended solids act as an abrasive and scrape the periphyton from the slide.)

Sloughing (As periphyton growth accumulates, some of the larger clumps slough off the glass slide.)

Substrate preference (Glass slides might not give a complete indication of the periphyton community at a sample site. Because of this possibility of missed species, natural substrates should be sampled during the long survey.)

Differences in amount of sunlight between sample sites (Sometimes this situation is unavoidable due to a stream's flowing through wooded areas. Light levels have a significant influence on biomass accumulation, chlorophyll concentration, community structure, species composition, or species structure).

Differences in orientation of samplers with respect to the pathway of the sun [Preliminary results of a recent study by the Ecology Branch, Chemical Systems Laboratory at Aberdeen Proving Ground, MD (unpublished) indicate this may have a significant influence on biomass accumulation.]

Vandalism (Samplers are sometimes stolen or damaged by vandals.)

Flooding (Samplers can be damaged or lost during floods.)

Freezing of samples for chlorophyll a determination may rupture cells, causing loss of contents, including chlorophyll a.

(3) Equipment Checklist Refer to Appendix C.

Artificial substrate sampler (for holding glass slides)
New, precleaned, unfrosted, standard glass microscope slides
2.5 x 7.6 cm (1 x 3 inches)
Stainless steel cable, 0.3 cm in diameter
Concrete block anchors
Cable cutters
Cable fasteners and crimping tool
One-gallon plastic bottle floats
Razor blade
Knife
Ice chest with ice
4 percent formalin (Appendix E)
Filtered water from the sample site
118 ml (4 oz) brown sample bottles with labels

d. PREPARATION FOR TAXONOMY AND COUNTING (SHORT AND LONG SURVEYS)

In preparation for counting, it is necessary to identify all the diatoms present in a sample to the lowest possible taxonomic level (preferably species).

(1) Procedure

(a) Diatom cleaning

Pour a periphyton sample preserved in 25 ml of 4 percent formalin into a 100 ml beaker.

Add 25 ml of concentrated nitric acid and approximately 0.5 g of potassium dichromate to the sample.

Boil the sample in a ventilated exhaust hood until the volume has decreased to about 25 percent (12.5 ml) of the original sample plus acid.

If organic material remains (the suspension is cloudy), repeat the previous two steps.

Pour the sample into a centrifuge tube and centrifuge at 170 gravities[1] for 20 minutes.

[1]The equation for calculating gravities (relative centrifugal force) is: $0.000001118 \times r \times N^2$; where r = rotating radius [centimeters (distance from center of centrifuge head to halfway down one of the centrifuge tubes)]; and N = rotating speed of centrifuge (revolutions per minute).

Decant the supernatant, add approximately 12.5 ml of distilled water, and resuspend the periphyton.

Repeat the previous two steps two more times.

Pour the resuspended sample into a thoroughly rinsed (six to ten times with distilled water) graduated cylinder and record the volume[1] on the periphyton bench sheet (Figure 4-2).

(b) Slide Preparation

With a 1-ml pipette, place 1.0 ml, 0.1 ml, and 0.01 ml of the sample on three separate, 22 mm coverslips. Be sure to keep track of each coverslip.

Where necessary, add distilled water to bring the total volume on the coverslip to 1.0 ml. Warm to dryness (do not boil) on a slide warming tray.

Place clean microscope slides on a warming tray and place two to three drops of Hyrax^{R2} mounting medium [refractive index (R.I.) = 1.66] or equivalent on the slides.

Place dried coverslips (R.I. = 0.05) (dried periphyton material down) over the media and mark the slide to indicate the appropriate sample volume used.

Heat gently to drive off the solvent. Press the coverslip down with pencil eraser to force out any excess mounting medium.

The slides are now prepared for counting. On each coverslip, count the number of frustules in a representative field[3] under an oil immersion microscope (1000X magnification). The desired result is four to five whole diatom frustules per field. If this result is not attained with the three sample volumes used, the actual counts obtained can be used to determine the proper volume of resuspended periphyton sample to use in the above procedure. In any case, record the final volume[3] of sample used on the long survey bench sheet (Figure 4-2).

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[1]Volumes will be recorded for the long survey only.

[2]Custom Research and Development Inc., Richmond, CA

[3]The representative field is identified on the high-dry (400X magnification) setting.

SAMPLE SITE_____ DATE COUNTED_____

LOCATION_____
(Include township and range, if possible)

COUNTY_____ STATE_____

COLLECTION DATE_____ BY_____

COLLECTION METHOD_____ DAYS INCUBATED ON ARTIFICIAL SUBSTRATE_____

Final Volume of Resuspended Periphyton _____ ml (A)

Volume of Final Sample of Resuspended Periphyton Placed on Coverslip (4-5 diatom frustules/field) _____ ml (B)

Total Count _____ (C)

(A)/(B) _____ (D)

Number of Fields Counted _____ (E)

$$\frac{(C) \times (D)}{(E)} (491.34) = \text{\_\_\_\_} \frac{\text{Diatoms}}{\text{cm}^2 \text{ of Substrate Area}}$$

Species	Number of Organisms

Figure 4-2. Periphyton Bench Sheet for Long Survey

(2) Equipment Checklist Refer to Appendix A.

Concentrated nitric acid (technical grade)
100 ml beaker
Potassium dichromate (technical grade)
Hot plate
Centrifuge with 20 ml centrifuge tubes
25 ml graduated cylinder
1.0 ml and 0.1 ml pipettes
Slide warming tray
Exhaust hood
Microscope equipped with 40x high-dry and 100x oil-immersion objectives and 10x eyepieces. Phase contrast equipment is highly desirable
Hydrax^{R} mounting medium, R.I. = 1.66
New, precleaned, unfrosted, standard glass microscope slides 2.5 x 7.6 cm (1 x 3 in)
New, 22 mm square Number 1 coverslips, R.I. = 0.05
Immersion oil
Tally counter
Periphyton bench sheets (Figures 4-1 and 4-2)
Pencil with eraser

e. DIATOM COUNTING FOR SHORT SURVEY

Because it is difficult to measure the area of natural substrate sampled during a short survey, the data obtained are qualitative.

(1) Procedure

Place the slide on the microscope stage and with an oil-immersion objective (100x) identify (refer to Appendix F) and count all diatoms in a single transect across the square coverslip. Record the results on a short survey periphyton bench sheet (Figure 4-1).

Determine each species which accounted for more than 10 percent of the total number counted and subtract these numerically dominant species from the total.

Divide this result into the appropriate number[1] obtained from Table 4-1[2] in order to identify the number of transects to be viewed.

[1]Derived from the site characteristic factors (i.e. hardness, salinity, organic enrichment) determined early in the survey and recorded on the periphyton bench sheet for short survey (Figure 4-1).

[2]Hohn, M.H., "Determining the Pattern of the Diatom Flora," Journal Water Pollution Control Federation, 33:48-53, 1961.

Table 4-1. Factors for Calculating the Total Number of Coverslip Transects to be Viewed Based on Water Quality of the Sample Site

Description of Water[1]	Healthy With No Evidence of Organic Pollution	Healthy But Organically Polluted	Polluted (Toxic)
Fresh, soft water	8000	5000	1500
Fresh, hard water	5000	3700	1500
Brackish water	8000	4000	1500

View the additional transects, recording species not already listed (no counting is performed).

When all transects have been viewed, construct a truncated normal curve according to the method of Hohn[2]. Patrick[3] gives details on the analysis of these curves.

(2) Sources of Error

See page 4-12.

f. DIATOM COUNTING FOR LONG SURVEY

The data are used to calculate diversity indices, similarity indices, and the summary of ecological characteristics of the community by using Lowe's ecology matrix[4]. In addition, increases in standing crop over time (growth rate) can be determined.

[1]Natural, nonmarine waters are divided (on the basis of salinity) into fresh (0-999 mg NaCl/ℓ) and brackish water (1000-5000 mg NaCl/ℓ). Fresh water can be further separated on the basis of hardness into soft (0-75 mg equivalents $CaCO_3$/ℓ) and hard (76-up mg equivalents $CaCO_3$/ℓ).

[2]Hohn, M.H., "Determining the Pattern of the Diatom Flora," Journal Water Pollution Control Federation, 33:48-53, 1961.

[3]Patrick, R., M.H. Hohn, and J.H. Wallace, "A New Method for Determining the Pattern of the Diatom Flora," Notulae Naturae, No. 259, 1954.

[4]Environmental Protection Agency, Washington, DC 20460. Environmental Requirements and Pollution Tolerances of Freshwater Diatoms, R. Lowe, 1974.

(1) Procedure

Place the slide on the microscope stage and with an oil-immersion objective (100x) identify (refer to Appendix F) and count all diatoms found in random fields. (Use a random selection system, page 11, Volume 7.) Count no less than 30 random fields and 120 cells (i.e. more than 30 fields may have to be counted to obtain 120 cells total. However, if 120 cells are counted in less than 30 fields, counting must continue until 30 fields have been examined).

On the periphyton bench sheet (Figure 4-2), record the number of each species (or other taxa) encountered and the number of microscope fields viewed.

Use the equation in Figure 4-2 to determine the number of diatoms (cells) per square centimeter (cm^2) of substrate area (glass slide).

(2) Sources of Error for Short and Long Surveys

Incorrect identification (Because of the lack of taxonomic reference material or the observer's inexperience, incorrect identifications may be made. If the incorrect identifications are consistent, this error is unimportant where the data are to be used for those indices, truncated normal curves, etc., where the actual species name is not important. When analyzing the ecology of the species present at a station, incorrect identifications can lead to invalid inferences. The potential for this error can be reduced by verification of the identifications by an expert and by having sufficient taxonomic literature available.) Retain voucher specimens of each taxon.

Non-random distribution on slide (Because both counting methods are based on the assumption of a random distribution, any deviation can lead to errors. Therefore, use extreme care in preparing the mount.)

Dead or fossil cells sticking to slide in sampler (Because the diatoms are acid cleaned, there is no way of determining which diatoms were alive at the time of sampling. Because the only diatoms of interest are the ones living at the sample site, the data can be affected by inadvertent counts of dead or fossil cells, but it is difficult to estimate by how much.)

(3) Equipment Checklist Refer to Appendix A

Microscope with 100x oil-immersion objective and 10x eyepiece
(Phase contrast equipment is highly desirable.)
Periphyton bench sheets, Figures 4-1 and 4-2

Taxonomic references[1]
Tally counter

g. CHLOROPHYLL a

Chlorophyll a concentrations (corrected for pheophytin a) provide estimates of algal biomass (all photosynthetic algae contain chlorophyll a) and net primary productivity.

(1) Procedure

Chlorophyll a concentration is determined with a fluorometer. Because the determination is rather complicated and requires a specialized piece of equipment, it normally will be contracted out to a special laboratory. A detailed description for the procedure, useful for writing a scope of work or for performing the determinations if qualified personnel are available, can be obtained through the Life Sciences Division, US Army Dugway Proving Ground, UT. The following is a description of the procedure for preparing the sample for shipment to the laboratory making the determinations.

Remove one of the three bottles (page 4-5) from the ice chest.

Filter the contents with filtered sample site water through the glass fiber filter[2] (in a Millipore[R][3] or similar apparatus).

Place a paper spacer from the box of glass fiber filters over the material trapped on the glass fiber filter. Wrap the resulting "sandwich" in aluminum foil, apply masking tape, and use waterproof ink to write the sample identification number (refer to Appendix A) on the tape. Use the shipping method for frozen samples (Appendix B) to ship the samples to the laboratory that will perform the chlorophyll a determination. The determinations must be made within 30 days.

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[1]Local references as appropriate (refer to Appendix F). One general reference is: Patrick, R. and C.W. Reimer, The Diatoms of the United States, Vols 1 and 2, Monograph of Academy of Natural Sciences of Philadelphia, No. 13, 1975.

[2]Reeve Angel, Catalogue No. 984H, Arthur H. Thomas Co., Vine St. at 3rd, P.O. Box 779, Philadelphia, PA 19105.

[3]Millipore Corporation, Bedford, MA 01730.

(2) Sources of Error

Degradation of chlorophyll a in the sample (This may occur for two reasons: (1) strong light and (2) enzymatic degradation. Therefore, samples should be kept in the dark and frozen.)

(3) Equipment Checklist Refer to Appendix A.

Glass fiber filters (effective pore size: 0.045 micrometer)
Vacuum pump
Millipore^{R} apparatus

h. ASH-FREE DRY WEIGHT

The ash-free dry weight (afdw) of the periphyton is an indicator of total periphyton biomass. This includes algae, fungi, bacteria, protozoa and some metazoa. It is used with the chlorophyll a data to calculate the autotrophic index[1] (A.I.), by which the health of the community can be estimated (A.I. >100 indicates organic enrichment).

(1) Procedure

Dry crucibles in drying oven, cool in dessicator, and weigh. Repeat the constant weight[2]. Record weight on data sheet (Figure 4-3).

Pour the remaining sample, (page 4-4), into the tared crucible and place in a drying oven (100°C) and repeat the above step to constant weight. Record weight on data sheet (Figure 4-3).

Place the crucible with the dried sample in a muffle furnace at 500 ±50°C for one hour.

[1]US Environmental Protection Agency, Cincinnati, OH 45268. "Periphyton", in Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents, Environmental Monitoring Series, Jul 1973.

[2]Dry crucibles and samples until no further weight loss can be detected by weighing each sample approximately every 12 hours until there is no more than ±0.05 mg difference in weights between successive samples.

SAMPLE SITE_____ DATE ASSAYED FOR ASH-FREE DRY WEIGHT_____

LOCATION_____
(Include township and range, if possible)

COUNTY_____ STATE_____

COLLECTION DATE_____ BY_____

COLLECTION METHOD_____

METHOD OF PRESERVATION_____ DAYS INCUBATED ON ARTIFICIAL SUBSTRATE_____

Ash-free Dry Weight

Area of Slide Scraped 37.5 cm^2

Dry Weight of Empty Crucible _____g (A)

Dry Weight of Crucible Plus Sample _____g (B)

Ash-free Dry Weight of Crucible Plus Sample _____g (C)

$$\frac{(B) - (A)}{37.5\ cm^2} = \_\_\_\_\_ g/cm^2 \text{ (Dry Weight)}$$

$$\frac{(C) - (A)}{37.5\ cm^2} \quad \_\_\_\_\_ g/cm^2 \text{ (Ash-free Dry Weight)}$$

Figure 4-3. Ash-free Dry Weight Data Sheet

Cool in a dessicator and wet with a few drops of distilled water to replace water of hydration.

Dry the crucible with sample in a drying oven (100°C) to constant weight as before. Record data on the afdw data sheet (Figure 4-3).

Calculate the dry weight and afdw of the sample using the equation of the afdw data sheet (Figure 4-4).

(2) Sources of Error

Caddisfly larvae cases (Unless caddisfly larvae cases are removed from the sample, an overestimate of periphyton biomass will be obtained.)

(3) Equipment Checklist Refer to Appendix A.

Muffle furnace (capable of heating to 500°C)
Drying oven (capable of heating to 105°C)
Dessicator with silicone stopcock grease and dessicant
Balance (accurate to 10^{-4} g)
Crucibles
Data sheets, Figure 4-3

i. SOURCES OF FURTHER INFORMATION

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NOTES

CHAPTER 5 - AQUATIC MACROINVERTEBRATE SAMPLING

CONTENTS

a. INTRODUCTION

As discussed in this chapter, aquatic macroinvertebrates are those invertebrates which live all or part of their life cycle within or upon submerged substrates and are retained by a U.S. Standard Number 30[1] sieve. The macroinvertebrate fauna in freshwater includes clams, mussels, crustacea, insects (adult and immature), flatworms, roundworms, and annelids. They are important members of the food web and their condition is reflected in the condition of fish populations.

Aquatic macroinvertebrates have limited mobility, longer life spans than periphyton, and their community structure is sensitive to stress. Various macroinvertebrate species have different pollution tolerances and environmental requirements. These characteristics make macroinvertebrates useful biological indicators.

Aquatic macroinvertebrate habitats include bottom sediments, logs, debris, pilings, and riffles. Other aquatic macroinvertebrates are free-swimming forms. Collection methods vary with the habitat type sampled.

[1] 0.595-mm sieve openings (28 meshes/in.)

b. GRAB SAMPLING OF SOFT SEDIMENT

Soft sediments are found in areas of low current velocity. They are sampled in replicate with a "grab" (e.g. an Ekman grab) which removes a portion of the substrate, including the macroinvertebrates living there.

(1) Procedure

Open grab sampler jaws.

Lower grab sampler to substrate.

(a) For deep water, allow sampler to fall freely.

(b) For shallow water, push the sampler into the sediment to a depth between 6 to 15 centimeters (2 to 6 inches).

Release the spring on the sampler jaws.

(a) For deep water, use a messenger to release the spring.

(b) For shallow water, push plunger rod in the grab handle.

Pull sampler to the surface and empty the contents into a plastic wash pan.

(a) Examine the jaws of the grab sampler before opening it to be certain the jaws closed properly. If not, discard the sample away from the area sampled.

(b) Also examine the screen top of the sampler to be sure that the sampler was not buried in the mud. If so, discard the sample away from the area sampled.

Pour contents of the pan into a Wildco[R1] sieve bucket and away from the area sampled. Wash the mud through the screen by agitation.

(a) Do not push the mud through the screen because this will damage the organisms.

(b) If large clods of granular clay or detritus are present, these may be broken up carefully by hand.

[1]Wildco, 301 Cass St., Saginaw, MI 48602.

Use a wash bottle (containing 70 percent isopropyl alcohol with rose Bengal dye) to wash the contents of the sieve into a glass quart jar.

(a) Cover the material with isopropyl alcohol containing rose Bengal dye, screw the lid on tightly, and gently roll the jar to expose all material to alcohol.

(b) Label a waterproof-paper tag with the sample site and replicate numbers as in Appendix A; place the tag inside the jar and screw on the lid.

(c) Record the same information on the jar with a waterproof marking pen.

(d) Record the same information in the survey log (Appendix A).

Collect five samples from each sample site for preliminary sampling. After the preliminary information is obtained, proper sample size can be estimated following the method of Weber[1].

Hold the samples at least 48 hours before sorting so that the dye can be absorbed properly. Organisms will be stained pink to red and easily distinguished from detritus.

Refer to the procedures for sorting preserved samples, page 5-6.

(2) Sources of Error

Variations in the quantity of material collected (This can be reduced by selecting sample sites with sediments of similar texture and consistency so that the sampler penetrates each sediment to approximately the same depth.)

Submerged vegetation (Should be avoided)

[1]National Environmental Research Center, Environmental Protection Agency, Cincinnati, OH 45268. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents, Weber, C.I., 1973.

(3) Equipment Checklist Refer to Appendix C.

Brass Ekman grab, with handle for shallow water or messenger for deep water
Sieve bucket with Number 30 mesh screen bottom
Glass quart jars with lids
Isopropyl alcohol (seven parts technical grade alcohol, three parts distilled water) with 15 mg/ℓ rose Bengal dye
Plastic wash bottle

c. SAMPLING RIFFLES

Riffles occur where water is shallow and flow is rapid. Aquatic macroinvertebrates are sampled by agitating the substrate and allowing the water flow to wash them into the net of a portable invertebrate box sampler[1].

(1) Procedure

Assemble the metal box of the sampler, making sure that the net extends freely behind the frame. If the water is deeper than the height of the net frame, use a dip net or kick screen instead.[2]

Place the foam pad of the sampler on the rocky bottom of the riffle with the net trailing downstream.

Agitate the rocks and sediment inside the box. Strands of algae, silt, and macroinvertebrates will be swept downstream into the net.

Pick up the rocks inside the frame and wash all detritus and mud into the net. If necessary, use a soft brush to gently scrub the rocks.

Wash the material in the net to the end of the net.

Remove the net from the box and empty the contents into a jar.

(a) With forceps, pick the debris and organisms from the net and drop the organisms into the sample jar.

[1]Ellis-Rutter Associates, P.O. Box 394, Douglassville, PA 19518.

[2]The kick screen is pushed into the sediment and the bottom material upstream is agitated by a second person. Macroinvertebrates dislodged from the substrate are swept into the screen.

(b) Add 70 percent isopropyl alcohol containing rose Bengal dye to the jar until the sample is covered. Label the jar with the sample site number and replicate number as in Appendix A.

Refer to the procedures for sorting preserved samples (page 5-6).

(2) Sources of Error

Uneven or hard-bottom sediments

(3) Equipment Checklist Refer to Appendix C.

Portable invertebrate box sampler
Dip net
Kick screen
Chest-high waders
Plastic wash bottle
Soft brush
Forceps
70 percent isopropyl alcohol (seven parts isopropyl alcohol, three parts distilled water) with 15 mg/ℓ rose Bengal dye

d. SAMPLING ARTIFICIAL SUBSTRATES

Artificial substrates are used to collect free-swimming organisms and when natural habitats are not available. The artificial substrate used is the Hester-Dendy multiple-plate sampler[1]

(1) Procedure

Suspend sampler containing five or more plates from a buoy, fallen tree, or other fixed object, as available.

(a) All substrates should be under water during the entire period of exposure.

(b) The same type of fixed object must be used at all sample sites. Attaching the substrates to buoys guarantees that the substrates will remain a constant depth below the surface. Unfortunately this method exposes their location to vandals.

After a two-week exposure (a four-week exposure in the winter) collect the sampler in a sieve bucket.

[1]F.E. Hester and J.B. Dendy, "A Multiple-plate Sampler for Aquatic Macroinvertebrates", Trans. Amer. Fish. Soc., 91:420-421, 1962.

Place the sampler in a cloth bag. With a waterproof marker, label with sample site number, replicate number, and date as in Appendix A.

Wash the contents of the sieve bucket into the cloth bag with the sampler[1]. Close the bag with a wire twist tie or string. Place the bag in a covered bucket with ice.

Scrape the samplers within 48 hours. Between collecting and scraping, refrigerate at 4°C.

(2) Sources of Error

Scouring the surface (refer to Chapter 4)

Vandalism (refer to Chapter 4)

Substrate preferences of the organisms (refer to Chapter 4)

Seasonal variations in the colonization rate of aquatic macroinvertebrates (Only compare those plates taken at the same time of year.)

(3) Equipment Checklist Refer to Appendix C.

Hester-Dendy multiple-plate samplers
Sieve bucket with Number 30 mesh screen bottom
Cloth bags (20 cm x 20 cm) with wire twist ties or string
Plastic wash bottle
Forceps
Buoys, anchor, and rope or stainless steel cable
Refrigerator

e. SORTING AQUATIC MACROINVERTEBRATE SAMPLES

Aquatic macroinvertebrates collected by the above techniques are retained as preserved (grab and riffle) or live (artificial substrate) samples. The sorting method used will depend upon the manner of retention.

The macroinvertebrates in the sample must be sorted from the mud, sticks, leaves, and other detritus that remain after the preliminary

[1]The organisms must be removed from the sampler before they are preserved. Otherwise the masonite sampler plates will absorb the preservative which will inhibit colonization of the plates during future sampling. The plates should only be washed with water. Samplers which have been subjected to toxic pollutants (such as oil) should not be reused.

washing in the field. Before sorting a sample you should know the many shapes and sizes of the organisms that you will be separating. A guide and description of these are included in Appendix G.[1]

(1) Procedure

Preserved samples

With tap water, thoroughly wash (six to 10 times) a sieve pan (Number 30 or smaller mesh) and white enamel sorting pan.

Apply a length of tape to bench top, cut into label-sized lengths, label, then apply to bottle. Place a piece of tape (such as Time^{R}) on a (59- or 118-ml) jar and write the sample site number, replicate number, and date on the tape. Fill the jar with 70 percent isopropyl alcohol. If necessary, write a new label on bond paper and place it inside the jar. Discard the old label.

Select a small portion of the sample for sieving (approximately 20 ml).

Gently wash the sieve at an outside faucet or wash in a sink over a pan. DO NOT WASH DIRT DOWN THE DRAIN.

Place the washed material in water in a white enamel pan and pick all undyed animal specimens and red-dyed material (except cladocerans, which are generally planktonic rather than benthic, refer to Appendix G). Preliminary sorting may also be accomplished by floating the organisms in a saturated NaCl solution.

Place all organisms in the jar filled with 70 percent alcohol.

You are finished sorting when all material in the pan has been examined and a five-minute additional search under a 10x magnification produces no more organisms.

Discard debris in a dumpster or other appropriate garbage container.

Repeat the above seven steps until the entire sample is sorted.

If you interrupt the sorting, place a waterproof label (marked the same as the label in the jar) in the pan so that it is not confused with other samples.

[1]Needham, J.G. and P.R. Needham, A Guide to the Study of Freshwater Biology, Holden-Day, Inc., San Francisco, CA, 1962.

Each jar contains the invertebrates collected from one sample. At this time, make identifications to the lowest taxonomic level feasible. Separate representative specimens for taxonomic verification by macroinvertebrate taxonomists (refer to Appendix F). Use the methods of Appendix B to ship the specimens. Record each sample on a macroinvertebrate bench sheet (Figure 5-1).

Live samples

Because the predator/prey ratio can change if the samples are left unattended for long, as soon as possible after collecting the sample, empty contents of plastic bag into a white enamel pan. Separate the sampler plates and with a spatula, scrape all organisms on sides and edges into the pan. Wash each plate with tap water, collecting the wash water in the pan.

(a) If the water is clear after all plates are scraped and washed, proceed with the sorting. If the water is cloudy after all plates are scraped and washed, pour the contents of the pan through a Number 30 sieve.

(b) Backwash the material from the sieve pan into the enamel pan. The depth of water in the pan should not exceed 3 cm.

(c) Add Calgon^{R} to the water to help break up the clay particles. Because the Calgon^{R} will make the water cloudy, repeat steps (a) and (b) above.

Using forceps or a curved probe, pick all hard-bodied macroinvertebrates from the tray. Using an eye dropper, pick up soft-bodied macroinvertebrates. Place macroinvertebrates in a labeled (p 5-6) 59 ml (2-ounce) glass jar containing 70 percent isopropyl alcohol.

(a) Use the forceps or probe to move the detritus in the pan. Use a systematic approach, searching small, adjacent sections of the pan.

(b) You are finished when all material in the pan has been examined and a five-minute additional search with 10x magnification produces no more organisms.

Each jar contains the macroinvertebrates collected from one sample. At this time make identifications to the lowest taxonomic level feasible. Separate representative specimens for taxonomic verification by macroinvertebrate taxonomists (refer to Appendix F). Use the methods of Appendix B to ship the specimens. Record the results of each sample on a macroinvertebrate bench sheet (Figure 5-1).

SAMPLE SITE_____ METHOD OF PRESERVATION_____
REPLICATE NUMBER_____ DATE SORTED_____
COLLECTION DATE_____ BY_____
COLLECTION METHOD_____

Total Number of Organisms_____
Total Number of Taxa_____
Total Organisms by Weight_____

T[a]	TAXA	L[b](N)[b]	P[b]	TOTAL		T[a]	TAXA	TOTAL
	Diptera						Crustacea	
	Trichoptera						Hirudinea	
	Plecoptera						Nematoda	
	Ephemeroptera						Bivalvia	
	Odonata						Gastropoda	
	Neuroptera						Other	
	Hemiptera							
	Coleoptera							

[a]Initials of taxonomist in this column
[b]L = larval; N = nymphal; P = pupal

Figure 5-1. Aquatic Macroinvertebrate Bench Sheet

(2) Sources of Error

Failure to remove all specimens from the preserving jar, sampling device, and sieve screen result in incorrect specimen numbers.

(3) Equipment Checklist

Jar and label for each sample
Spatula
Forceps
Probe
White enamel pan
Sieve pan (Number 30 mesh)
Calgon^{R}
70 percent isopropyl alcohol (seven parts isopropyl alcohol, three parts distilled water)
10x magnification lamp or dissecting microscope
Tally counter

f. SOURCES OF FURTHER INFORMATION

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NOTES

CHAPTER 6 - FISH SAMPLING

CONTENTS

a. INTRODUCTION

Because fishing is an important public activity and fish are highly sensitive to chemical and physical changes in their environment, a decline in fishing success may incite the public to accuse local facilities of polluting the environment. This is one reason why fishes should be monitored. However, there are three limitations to fish surveys. First, changes in fish communities in a specific area are caused not only by pollution but also by life history requirements. Thus a detected change in the composition of a fish community after the discharge of a pollutant may in reality be related to reproduction or feeding movements. Second, fish are extremely mobile so they can rapidly repopulate an area even after an extensive fish kill. Therefore, providing dead fish are not detected, the effect of a toxic spill or a slug of effluent may not be detected in the fish community. Finally, fish surveys can be costly (but not as costly as lawsuits) because of the need for large samples, frequent sampling, and specialized sampling gear.

In general, natural fish communities can be monitored by studying two community parameters and six population parameters: (1) species present, (2) relative and absolute abundance, (3) size distribution, (4) growth rate, (5) success of reproduction, (6) general health, (7) incidence of disease and parasitism, and (8) palatability, respectively. In addition fishes are studied further in the laboratory for (9) behavior (e.g., ventilatory movement, erratic swimming), (10) mortality, (11) bioaccumulation of organic compounds and metals, (12) thermal tolerance, and (13) histopathological examination of various tissues.

This chapter discusses ways to perform the first seven of the above monitoring methods.

Fish are collected by active methods (seines, trawls, electrofishing, chemicals, and hook and line) or passive methods (hoop nets, gill nets, trammel nets, and fish traps). Each method includes a bias because it is selective for certain fish species. Two collecting methods will be described below: the seine and the hoop net. References to other methods are included at the end of this chapter. Only the less complicated methods will be described below.

Most state fishery agencies require a permit to collect fish for scientific research. Often the agency has previously collected information available. Cooperative efforts with local, state, and federal agencies are often more efficient and economical than unilateral surveys.

b. USING FISH SEINES[1]

Fish seines are used to provide estimates of species composition and relative abundance of fish populations in a shallow water area. They also are used to obtain specimens for examination and analysis.

(1) Procedure

Hand and beach seines are available in a variety of lengths, widths and mesh sizes. Each net is essentially a strip of strong netting hung between a cork or float line at the top and a strong heavily-weighted lead line at the bottom. Both ends are lashed to poles. As the net is towed, the netting forms an open bag. In a stream, two persons can pull a 3- to 4-m long seine upstream over a desired sample site. On the shoreline of lakes or slow flowing rivers, a 15- to 30-m long seine is used. One end is held on the shoreline and the other is stretched out perpendicular to the shoreline. The net is then rotated about the end on the shoreline until it is pulled parallel to the shoreline. The net is then beached rapidly by pulling the lead and float lines onto the shoreline together.

(2) Sources of Error

Schooling fish (Intercepting a school of fish will result in that species appearing more dominant than normal.)

Juvenile fish often are difficult to identify.

[1]The bottom should be free of trash and other debris which would snag the net.

(3) Equipment Checklist Refer to Appendix C.

Seine
Waders or hip boots

c. USING HOOP NETS

Hoop nets are used to provide estimates of species composition and relative abundance of fish populations from swamps, backwaters, or other areas where a seine is impractical because of submerged obstacles. Snapping turtles can be abundant in such areas, so use caution. The proper technique for handling snapping turtles can be found in A Field Guide to Reptiles and Amphibians[1].

(1) Procedure

Hoop nets are cylindrical nets held open by wooden hoops. They have at least one internal funnel. Hoop nets can be obtained with hoops up to 1.8 m in diameter, but small hoops 1.2 m or less are most useful for fish surveys. Hoop nets are placed where fish move in predictable directions. The net may be suspended on its side or vertically if the water is deep enough. They are baited with cheese, cotton cake, chicken parts, or pieces of dead fish suspended inside the net in a porous bag. After the hoop net is baited, it is tied to wooden stakes for support. Fish enter the net through the funnel(s) to get the bait.

(2) Sources of Error

Bait used (Bait may only attract selected species of fish.)

Size of hoop nets (Very large fish cannot enter the funnel and small fish swim through the mesh.)

Predators (Some fish in the net may be eaten by turtles or predatory fish before the sample is recovered.)

Vandalism (Place the nets where they are not easily noticed.)

[1]Conant, R., A Field Guide to Reptiles and Amphibians. Peterson Field Guide Series, Houghton Mifflin Co., Boston, MA, 1958.

(3) Equipment Checklist Refer to Appendix C.

Hoop net
Bait
Waders or hip boots
Wooden stakes
Porous bag
Heavy twine

d. PRESERVATION AND DATA COLLECTION

In some cases fish will be collected and preserved. More frequently they will be measured, identified, and released in the field.

(1) Procedure

Fix fish in a 10 percent formalin solution containing 3 g of borax (to maintain color) and 50 ml of glycerin/l (to maintain pliability). Specimens longer than 7.5 cm are slit on the side at least one-third the length of the body cavity to ensure fixation of the internal organs. Make the slit on the right side, because the left side is used for measurements. After fixation for one week, rinse the specimen in tap water and place it in 40 percent isopropyl alcohol for another 24 hours. For permanent storage, replace the used isopropyl alcohol with unused.

Each preserved specimen should include a standard label (Appendix A) bearing the sample identification number, locality, date, collector's name, and method of collection. Detailed observations about the collection should be recorded in the survey log.

Fish are measured on a fish board by laying the nose of the fish against the stop and measuring the length to the tip of the tail (total length).

At the laboratory, record pertinent information on the fish bench sheet (Figure 6-1) and the length and weight sheet (Figure 6-2).

(2) Equipment Checklist Refer to Appendix C.

10 percent formalin (one part 40 percent formaldehyde, three parts distilled water)
Glycerin
Borax
40 percent isopropyl alcohol (four parts technical grade isopropyl alcohol, six parts distilled water)
Fish board
Balance to weigh fish

SAMPLE SITE_____ COLLECTION METHOD_____

LOCATION_____
(Include township and range, if possible)

COUNTY_____ STATE_____

REPLICATE NUMBER_____ DATE SORTED_____ BY_____

COLLECTION DATE_____ BY_____

T[a]	Species	Total No. of Organisms
	Acipenseridae (Sturgeons)	
	_____	_____
	_____	_____
	Amiidae (Bowfins)	
	_____	_____
	_____	_____
	Lepisosteidae (Gars)	
	_____	_____
	_____	_____
	Salmonidae (Trout, salmon, whitings, and graylings)	
	_____	_____
	_____	_____
	_____	_____
	_____	_____
	_____	_____
	Cyperinidae (Carp, dace, minnows)	
	_____	_____
	_____	_____
	_____	_____
	_____	_____
	_____	_____
	_____	_____

Figure 6-1. Fish Bench Sheet

T[a]	Species	Total No. of Organisms
	Catostomidae (Suckers)	
	Ictaluridae (Catfish)	
	Cyprinodontidae (Killifishes)	
	Centrarchidae (Sunfishes and basses)	
	Percidae (Perches and darters)	
	Others	

[a]Initials of the taxonomist

Figure 6-1. Fish Bench Sheet

SAMPLE SITE_____ COLLECTION METHOD_____

LOCATION_____
(Include township and range, if possible)

COUNTY_____ STATE_____

REPLICATE NUMBER_____ DATE SORTED_____ BY_____

COLLECTION DATE_____ BY_____

Species	Length (cm)	Weight (grams)	Species	Length (cm)	Weight (grams)

Figure 6-2. Fish Laboratory Length and Weight Sheet

e. IDENTIFICATION OF SPECIES Refer to Appendix F.

Fish may be grouped or classified in many different ways. Grouping schemes have been based on ecological significance, feeding habits, and commercial importance. There are numerous regional manuals for the identification of freshwater fishes, but the best general reference is How to Know the Freshwater Fishes[1]. In addition, federal and state agencies can often provide assistance with fish collecting and identification guides.

f. SOURCES OF FURTHER INFORMATION

Carlander, K.D. Handbook of Freshwater Fishery Biology. Vol. I. Iowa State Univ. Press, Ames, IA, 1969.

Sources of Limnological and Oceanographic Apparatus and Supplies, American Society of Limnology and Oceanography, Special Publication Number 1, IX:i-xxxii, 1964.

Sinha, E.F. and C.L. Kuehne, "Bibliography of Oceanographic Instruments", Meteorological and Geoastrophysical Abstracts, American Meteorological Society, 14:1242-1298, 1589-1637, 1963.

U.S. Fish and Wildlife Service, Washington, DC 20036. "Partial List of Manufacturers of Fishing Gear and Accessories and Vessel Equipment," Fishery Leaflet 195, 1959.

Vibert, R. (ed). Fishing with Electricity - Its Application to Biology and Management. European Inland Fish. Adv. Comm. FAO, United Nations, Fishing News (Books) Ltd., London. 1967.

Weber, C.I., National Environmental Research Center, Environmental Protection Agency, Cincinnati, OH 45268, Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents, 1973.

[1]Eddy, S., How to Know the Freshwater Fishes, William C. Brown, Co., Dubuque, IA, 1957.

APPENDIXES

APPENDIX A - RECORD KEEPING

Survey Log

The log should be maintained in a bound notebook with consecutively numbered pages. Each entry should begin with the date and time of the survey described in the entry. It should end with the date and time the entry was made in the log. Always identify and describe the location of the survey (include township and range, if possible), preferably include a reference to a sample site number on a map (see below), in which case the map should be taped to the log book. Always identify those people accompanying you in the survey. Describe as much detail as possible - often seemingly insignificant observations may become important later on. Sign your name in the lower left corner of each page. All entries should be made with waterproof ink. Line out erroneous entries with a single line.

Survey Area Map

A map of the survey area is essential and each member of the team should have a supply of maps so that he can locate each major place and event accurately, including sample sites and locations of observations. U.S. Geological survey topographic map indeces, published for each state, Puerto Rico, the Virgin Islands, Guam, and American Samoa, are available free from the U.S. Geological Survey, Washington, DC 20244 or the Federal Center, Denver, CO 80225. Appropriate quadrangles may be ordered at minimal cost from agencies identified on these indeces. Most U.S. Army Facilities Engineering Directorates have all quadrangles for the installation. The quadrangles may be purchased at sporting goods stores in some areas. Usually, the most desirable scale is the one showing the greatest detail of the area.

Labeling Samples

Place each sample in a separate container along with a prenumbered waterproof tag[1]. If the sample will be used for chemical analyses, do

[1]These tags are prenumbered using a black, waterproof felt tip or permanent ink pen (waterproof labels are prepared in a similar manner). Place the tags on a ring in consecutive order.

not include the tag. Instead, place a strip of tape[1], with the same number as the tag, on the outside of each container.

Fill out two replicate standard labels (page A-3) for each sample. The identification number is the number you placed on the sample container. Attach one label to the log (above) and one label to the sample container. Beneath the label attached to the log, enter other pertinent data such as weight, organs sampled, and where shipped.

Standard Label

Figure A-1 is a series of standard labels. The page can be reproduced and the labels cut out for use. Explanation of entries is as follows:

"ID No." - The identification number used to mark the sample. It can include an identification number for the transect, sample site, replicate, and date.

"Collector" - Your name (also name other people present when the sample was collected).

"Coll Date" - The date the sample was collected.

"Coll Location-site" - The site where the sample was collected. Refer to a place name found on your map. For example: "Orr Springs" "20 km NNW Hatch Ranch" (Note: use metric system, km-kilometers and N, E, S, W for North, East, South, and West, respectively.).

"Sample Type" - Dredge, dip net, etc.

Photographs

Color and black-and-white photographs should be taken to record and substantiate conditions observed at the sample site. It is important to have some identifiable object in the picture to serve as a point of reference and as a scale to the area encompassed by the photo. The sun should be behind the photographer. Photographs taken between 1100 and 1400 hours give the best results. The use of a polarizing filter will reduce the glare of reflected light from the surface of water.

[1]The tape is prepared by sticking 2.5-cm wide strips of yellow Time^{R} tape or equivalent to a sheet of stiff plastic or wood and numbering short segments of the tape with the identification number. When numbering both the cardboard tag and tape, place a short line under each number to ensure the number is read right-side up. In the field, as needed, use a knife to cut the prenumbered tape segments from the sheet.

ID No________ Coll Location-site________
Collector________ County________
Coll Date________ State________
Sample Type________

- - - - - - - - - - - - - - - - - - - -

ID No________ Coll Location-site________
Collector________ County________
Coll Date________ State________
Sample Type________

- - - - - - - - - - - - - - - - - - - -

ID No________ Coll Location-site________
Collector________ County________
Coll Date________ State________
Sample Type________

- - - - - - - - - - - - - - - - - - - -

ID No________ Coll Location-site________
Collector________ County________
Coll Date________ State________
Sample Type________

- - - - - - - - - - - - - - - - - - - -

ID No________ Coll Location-site________
Collector________ County________
Coll Date________ State________
Sample Type________

- - - - - - - - - - - - - - - - - - - -

ID No________ Coll Location-site________
Collector________ County________
Coll Date________ State________
Sample Type________

- - - - - - - - - - - - - - - - - - - -

ID No________ Coll Location-site________
Collector________ County________
Coll Date________ State________
Sample Type________

- - - - - - - - - - - - - - - - - - - -

Figure A-1. Standard Labels

APPENDIX B - SHIPMENT OF SAMPLES

In general, the following will apply to material being shipped, however, check with the receiving laboratory to be certain that the procedure is compatible with their operation. In general, foam insulated coolers enclosed in cardboard boxes make the best shipping containers regardless of the method of preservation.

Shipping Method for Frozen Samples[1]

Unless arrangements are made to receive samples on the weekends, samples should be sent by Tuesday. Early in the morning wrap all frozen specimens in insulating material, then place them in the insulated coolers with at least 5 cm of dry ice between them and all sides of the cooler. Enclose two reproduced copies of each page (from the log) containing any data on the specimens being sent. Securely close all openings in the box with filament tape. Label boxes in bold red letters: "Frozen scientific specimens - do not store in warm areas", and "Upon arrival at airport call _____" (put the telephone number of the receiving laboratory in the blank space). Obtain a bill of lading from your transportation office and immediately take the boxes to the nearest air freight office and send them on the most direct flight to the airport nearest the receiving laboratory. Notify the laboratory by telephone that the specimens are on their way. Also provide the name of the airport, the airline, the flight number, the expected time of arrival, and the bill of lading number.

Shipping Method for Non-frozen Samples[1]

The only differences between this method and the method for frozen samples are that the space occupied by dry ice is filled with shock absorbing materials and the label is changed to read "Scientific specimens - store at room temperature", and "Upon arrival at airport call _____".

[1]Completely-filled water sample bottles break when frozen, also water samples shipped during winter may be frozen during transit. When possible, avoid glass sample containers and leave air space for expansion of the frozen sample. For non-frozen samples, mark the package with a warning against freezing.

APPENDIX C - GENERAL EQUIPMENT CHECKLIST[1]

Vehicle (preferable 4-wheel drive), boat, and trailer[2]
Well-lighted work area with running water, distilled water, adequate bench space, telephones and 110 V and 220 V outlets
Map of site (Appendix A)
Survey log (Appendix A)
Standard labels (Appendix A)
Data sheets
Books or keys for the identification of specimens (Appendix F)
Pen filled with indelible ink
Felt-tip, permanent ink (waterproof) marker
Collecting permits
Graduated cylinder (graduated in 0.1 ml)
Photography equipment
  Camera
  Film
  Lenses (telephoto and wide angle)
  Filters
Clip boards
Sample Containers
  Cloth bags with twist ties or strings
  (118 ml) brown glass sample bottles
  Whirl-Pak^{R} bags
  Various glass or plastic jars to 1.5 ℓ size
Shipping excelsior or shredded paper or dry ice
Shipping cartons (foam insulated coolers) with cardboard outer containers
Filament tape
Label tape
Masking tape
Bill of lading

[1]For the Federal Supply Catalog number of some of these items, see Appendix 1, U.S. Department of the Army, Methods of Preparing Pathologic Specimens for Storage and Shipment, TM 8-340, Sep 1963.

[2]Personnel operating vehicles, boats, and trailers must be qualified to do so.

APPENDIX D - DEFINITION OF TERMS

Biomass - The total amount of biological material in a given habitat or area; or, an expression dealing with the total weight of a given population of an organism.

Community structure - The relative abundance of organisms inhabiting a common environment.

Diversity index - Pertains to the variety of organisms within a given habitat. High diversity is characterized by a great variety of species, usually with relatively few individuals of each species. Low diversity is characterized by a few species with one or more clearly dominant.

Frustule - The silica shell of a diatom. The ornamentation, shape, and size of the frustule are key characteristics for diatom taxonomy.

Net primary productivity - The rate of accumulation of organic material in plant tissues. That is, the production of organic material less that used in respiration.

Periphyton - The aquatic community composed of diatoms and other algae, bacteria, fungi, and protozoa which are attached to or growing on existing substrates.

Pheophytin a - The breakdown product of chlorophyll a. Unless corrected, it will interfere with chlorophyll a analysis.

Sample site - The point on the body of water which is to be sampled. Correct placement of the sites is essential to every survey. They are usually in upstream and downstream areas from the discharge point, under similar flow, temperature, and light regimes.

Similarity index - A measure of how much alike the communities at two or more sample sites are. It is usually used in conjunction with diversity indices because two sample sites can have the same diversity index and not be composed of the same species.

Species structure or composition - The kinds and numbers of organisms inhabiting a common environment.

Standing crop - The quantity of living organisms present in an area or region at a selected point in time.

Substrate - The material upon or within which an organism is living. Existing substrates include trash, rocks, logs, sediment, algal mats, and macrophytes. Artificial substrates are those which are placed in the water for the sole purpose of providing an area for the organisms to grow, usually for ease of sampling or quantification.

Survey area - A general location on a body of water containing one or more sample sites.

APPENDIX E - PRESERVATION AND STORAGE TECHNIQUES

Buffered formalin for tissue preservation[1] is prepared as follows:

40 percent formaldehyde - 2.0 ℓ
Distilled water - 18.0 ℓ
Dibasic sodium phosphate - 130.0 g
Monobasic sodium phosphate - 80.0 g

Mix until solution is clear. Place specimens in containers so that there is one part specimen to four parts formalin.

Frozen samples should be handled as per the instructions of the receiving laboratory.

Physiological saline solution is 0.9 percent NaCl in distilled water.

[1]Refer to Chapter 2, Section III, paragraph 16 and 18, U.S. Department of the Army, Methods of Preparing Pathologic Specimens for Storage and Shipment, TM 8-340, Sep 1963.

APPENDIX F - IDENTIFICATION OF SPECIES

State or area animal and plant keys are used where available, for identifying species. Generally keys are available through local universities or state fish and game departments. Chapter VI, Data Sources, in Study of Ecological Classification and Inventory Manual[1] provides a comprehensive listing of regional keys. National guides to identification are found in Section V, Appendix B, Volume 2.

Even with the above aids, the team leader may determine that trained taxonomists are required for identification of some taxa. Examples of these taxa will be prepared as directed by the team leader and sent to the agency or taxonomist[2] designated by the team leader per instructions in Appendix D.

[1]U.S. Department of the Navy, Naval Facilities Engineering Command, Alexandria, VA 22322. Study of Ecological Classification and Inventory Manual, Oct 1977.

[2]The Registry of Systematics Resources and Services (RSRS). Association of Systematic Collections, Museum of Natural History, University of Kansas, Lawrence, KS 66045, Phone: 913 864-4867; FTS: 752-2312, is a data base that maintains a list of names, addresses, and phone numbers of specialists willing to provide a variety of services in the fields of plant and animal taxonomy.

APPENDIX G - CHARACTERISTICS OF COMMON AQUATIC MACROINVERTEBRATES

SEGMENTED WORMS
(PHYLUM ANNELIDA)

Worms comprise one of the largest groups of animals. They have adapted to many different habitats, including the oceans, soil, and freshwater. Many species are internal or external parasites of man and other animals. The Annelida are the so-called "higher" worms - those creatures characterized by many segments, a soft, muscular body wall covered by a thin cuticle, and a complete digestive system. Two classes of segmented worms are likely to be collected: Oligochaeta (aquatic earthworms) and Hirudinea (leeches).

Aquatic oligochaetes occupy a niche equivalent to that occupied by earthworms. They feed on bottom mud and mix it much as earthworms effectively mix the surface layers of soils.

Many aquatic oligochaetes have hemoglobin in their blood and are able to thrive in low concentrations of dissolved oxygen. Tubificidae, (sludgeworms), especially the cosmopolitan Tubifex tubifex, occur in dense waving masses in polluted waters devoid of predators.

Quantitative sampling of bottom mud with dredges will usually yield many specimens of oligochaetes. Individual specimens can be collected by rinsing the mud through sieves or strainers, or simply by placing the mud in a laboratory pan and allowing the worms to come to the surface of the debris. Masses of filamentous algae, when rinsed or washed out with a stream of water, will also yield specimens.

The Hirudinea (leeches), are predominantly a freshwater group, but some species are marine and numerous terrestrial species occur in the tropics. In the United States, leeches are common inhabitants of ponds, marshes, lakes, and streams, especially in the north. They may range from 0.5 cm to 45 m in length. Many leeches are predators and scavengers, a few are ectoparasites on fish and other vertebrates.

Leeches are characterized by a segmented flattened body, with the most notable feature being two suckers, one at each end. The anterior, or oral sucker, is usually smaller and surrounds the mouth. The caudal sucker is much larger and more powerful and has a central attachment pedestal.

Leeches require objects to which they can adhere and consequently they are rare on pure mud or clay bottoms. They are most common in shallow waters, seldom being found below a depth of 2 m. In such warm, protected waters, where plants, stones, and debris offer concealment, population densities can reach 700 leeches per square meter of bottom. Leeches are chiefly nocturnal, therefore superficial daylight

examination of a likely body of water may be disappointing. Leeches are collected by turning over rocks, logs, and debris in the shallows and using forceps to remove each leech.

CRUSTACEANS
(PHYLUM ARTHROPODA, CLASS CRUSTACEA)

Crustacea are one of the two chief classes of the Arthropoda, a highly successful group of animals found in virtually every ecological niche. Arthropods are characterized by a hard external skeleton and several to many jointed appendages. Crustacea are distinguished by two pairs of antennae, respiration by gills (rarely through the general body surface), and by paired, jointed appendages on most of the body segments (at least the anterior ones).

A group as large as the crustaceans contains a wide variety of animals. Aquatic crustaceans range from the tiny water fleas and fish lice (1 mm), through the fairy shrimps and aquatic sow bugs (2 - 10 mm), to the larger crayfish and freshwater shrimps (greater than 10 mm). Marine crustaceans include crabs and lobsters. Sow bugs or pill bugs are perhaps the best-known terrestrial member of this diverse group.

Fairy, Tadpole, and Clam Shrimps

These small crustaceans are confined to temporary ponds and pools, expecially during spring and early summer. They move along the bottom or swim or glide about, often upside down. Specimens may be collected by a coarse dip net.

Water Fleas (Cladocera)

These small, ubiquitous, aquatic crustaceans are familiar to almost everyone who has collected in freshwater habitats. Cladocerans are abundant nearly everywhere, except the most rapid streams and brooks and the most grossly polluted waters. They are of great importance in the aquatic food chain as food for young and adult fish.

Copepoda

Like the cladocerans, copepods are found in almost every type of freshwater environment. Although not as structurally diverse as water fleas, copepods have radiated into more aquatic niches. For example, some groups are parasitic on fish, often causing considerable damage in hatcheries. Free-living copepods are on an intermediate trophic level between plankton (bacteria, algae, and protozoans) and predators (chiefly fish). Although copepods constitute a major food item for fish, they generally are not as important in the food chain as Cladocera.

Cladocerans and copepods are easily collected with a fine tow net or dip net. Most species are collected by drawing nets through rooted aquatic vegetation or by lightly skimming the bottom.

Seed or Mussel Shrimp (Ostracoda)

Ostracods are widely distributed and abundant but seldom receive the attention devoted to Cladocera and Copepoda. They live in a variety of substrates, including rooted vegetation, algal mats, debris, sand, mud, and rubble. Ostracods are so small (usually <1 mm long) that they are frequently overlooked in bottom samples. They are difficult to classify because the body is enveloped in a tiny, opaque, bivalve shell.

Aquatic Sow Bugs (Isopoda)

Although most isopods are marine or terrestrial, about 50 species occur in North American freshwater habitats. They usually are restricted to ponds, small lakes, streams, and subterranean waters. It is very unusual to find two or more species in the same habitat. They seldom occur in open waters but remain hidden under rocks, vegetation, and debris. Isopods can be collected by hand-picking, washing out aquatic vegetation, or with a small dip net.

Scuds and Sideswimmers (Amphipoda)

Amphipods are primarily marine, but about 50 species are found in aquatic habitats in the United States where they more or less are confined to the substrate. Most are larger than the crustaceans discussed above, some species reaching lengths of 20 mm. Amphipods require an abundance of dissolved oxygen, hence they are not common in polluted waters. They can be collected with a dip net and by rinsing out masses of aquatic vegetation and bottom debris.

Crayfish and Shrimps (Decapoda)

The vast majority of decopods are marine but freshwater forms can be abundant. Decapods occur in underground waters, flowing waters, sloughs, swamps, bayous, ponds, shallows of lakes, and even in wet meadows where there is no open water. Specimens can be collected with dip nets, dredges, or seines.

INSECTS
(PHYLUM ARTHROPODA, CLASS INSECTA)

Insects include far more species than any other class. Most are terrestrial, but significant numbers have adapted successfully to aquatic habitats, where they often are the most common macroinvertebrates. In most groups, the immature stages are aquatic and the adults are terrestrial. Truly aquatic adults are found only in the beetles and the true bugs, but even here, the adults are winged and leave the water to fly to nearby lights at night or to migrate to another body of water. Eleven orders of insects are represented in aquatic habitats; those not discussed below are the Collembola (springtails), a small primitive group with a few species adapted for life on the surface film; the Hymenoptera (wasps, ants, and bees), the aquatic forms of which are specialized parasitic species adapted to prey on other aquatic insects; and the Lepidoptera (butterflies), which have only two truly aquatic genera.

Stoneflies (Plecoptera)

The immature stages of stoneflies are restricted to running water. The nymphs are generally predators capable of rapid movement. The head is somewhat flattened and the body depressed with hooked legs for movement and attachment on rocks in swift currents. The abdomen has two stiff filaments (cerci) which further characterize the group. Some species may be >5 cm long. The adults are found near water. In general, Plecoptera nymphs require an abundance of oxygen, which is why they are restricted to flowing waters. They are also sensitive to low concentrations of heavy metals. They occur in debris such as masses of leaves and algae and under stones. They can be collected with dip nets or box samplers. Stonefly nymphs are an important part of the diet of stream fishes.

Mayflies (Ephemeroptera)

Over 500 species of mayflies are known from the United States. Like stoneflies, all the immature stages are aquatic while the adults remain near water, often in huge numbers. Large, paired tracheal gills on the lateral or dorsal surface of most of the abdominal segments are the most characteristic feature, distinguishing mayfly nymphs from all other aquatic insects. In addition, three long, segmented, fringed, caudal filaments usually occur at the tip of the abdomen. Mayfly nymphs are often the most abundant insects in clean, cool streams. Species occurring in vegetation or stones can be collected with a dredge or dip net, or by washing out the vegetation in a container. For stream work a box sampler held in the current while rocks and debris upstream are agitated will retain many dislodged specimens.

Dragonflies and Damselflies (Odonata)

These familiar insects also have exclusively aquatic nymphs and terrestrial adults. Nymphs and adults are voracious predators; some of the larger nymphs prey on small fish. Dragonfly nymphs are more robust than most other aquatic nymphs. They are aggressive predators, capable of rapid movements by expelling water from the abdomen. Damselfly nymphs have delicate anal gills. Odonata nymphs may be grouped into climbers, sprawlers, and burrowers. Climbing nymphs can be collected by rinsing out masses of vegetation in a pan. Sprawling and burrowing forms are found by raking up bottom trash or by pulling a wire seive net through the surface layers of mud, sand, and debris. Odonates are relatively insensitive to dissolved oxygen concentrations. However, they are usually absent when pesticides, chlorine, and heavy metals are present in water.

Bugs (Hemiptera)

True bugs constitute a large and important order of insects. Eleven families are aquatic. Among the more common and better known families of aquatic bugs are the Gerridae (water striders), Notonectidae (back swimmers), Belostomatidae (giant water bugs), and Corixidae (water boatmen). Hemiptera are distinguished from other orders of insects by three characteristics: (1) the mouth parts are greatly modified to form a jointed, piercing, and sucking "beak"; (2) the anterior pair of wings are leathery at the base and membranous and crossed at the tips, while the second pair are entirely membranous; and (3) metamorphosis is simple and gradual. In aquatic forms, the nymphs and adults live in water, occassionally flying from one body of water to another. Nearly all aquatic Hemiptera are predators. They are collected in much the same way as described for other aquatic insects. Most hemiptera are air breathers. Nevertheless, they generally are intolerant of changes in water quality. Because they live within the water column, darting among vegetation, the surface, and sediments, estimates from sediment samples are not representative of their true abundance in the receiving waters.

Alderflies and Dobsonflies (Neuroptera)

Although very few Neuroptera species are aquatic, they are among the most striking of freshwater insects. Hellgrammites, the large larvae of dobsonflies, are prized fish bait. Their bodies are dorso-ventrally flattened with prominent, chitinized segments. The pinching mouth parts are strong enough to break the skin and cause severe bruises. They are very sensitive to poor water quality. Neuroptera are found along the margins of ponds and lakes and under and between stones in streams.

Caddisflies (Trichoptera)

Larvae of caddisflies are among the most unique of aquatic insects. The larvae construct elongate cases of sand grains, gravel, small pieces of leaves, grass, bark, or twigs. They live in the cases on the bottoms of shallow ponds and streams. Adult caddisflies are found near water.

Larvae can be collected from the substrate by dredge or net and then washed out of the drift, debris, or vegetation in a sieve or large pan. The case is used in species identification, therefore care should be taken to keep the case and larva together. Most species require water with an adequate supply of oxygen. However, some net-spinning (as opposed to case-constructing) caddisflies are abundant downstream of sewage outfalls. Caddisflies are one of the most important foods for fish.

Beetles (Coleoptera)

Beetles comprise the largest order of insects; more than a quarter million species are known. Many resemble the Hemiptera, however, the anterior wings (elytra) are parallel rather than crossed. Thirteen families live in aquatic habitats. Among the more common families of aquatic beetles are the Dytiscidae (predaceous diving beetles), Gyrinidae (whirligig beetles), Hydrophilidae (water scavenger beetles), and Elmidae (riffle beetles). Many beetles breathe air or capture air bubbles at the surface. As a result, the abundance and diversity of beetles are generally unrelated to slight changes in dissolved oxygen content. Elmidae are a major exception, being so dependent on high concentrations of dissolved oxygen that they are restricted to riffles and fast-flowing water. Usually both immature stages and adults are found in the water. Coleoptera are found in stream, rivers, ponds, and protected bays of lakes. They may be collected easily with a dip net or by washing out vegetation. Many common species are often overlooked by the general collector because of their small size, inconspicuous coloration, and secretive habits.

Flies, Mosquitoes, and Midges (Diptera)

Eighteen families of freshwater Diptera are represented in North America. Among the major families are Tipulidae (crane flies), Psychodidae (moth flies), Culicidae (mosquitoes), Simuliidae (black flies), Chironomidae (midges), Ceratopogonidae (biting midges), Tabanidae (horse flies), Syrphidae (flower flies), Sciomyzidae (marsh flies), and Ephydridae (shore flies).

The adults of many aquatic species feed on blood. Because they harbor and transmit pathogenic organisms which cause such diseases as malaria, elephantiasis, yellow fever, encephalitis, dengue fever, and some cattle fevers, they are of great significance.

Without exception, aquatic species whose larvae live in water have adults which are terrestrial. Larvae may be collected by dredging, sieving, dip netting, or washing. Diptera generally are associated with foul water. However, some species are very sensitive to degradation while others are sensitive to enhancement of water quality. Generally, as the speed of the current increases, the variety and abundance of Diptera decrease. Where possible, evaluations should be based on species level identification, which often requires a specialist for each major family. Some confusion can be resolved by rearing the larvae in small vials half filled with water and plugged with cotton.[1]

[1]Merrit, R.W. and K.W. Cummins, An Introduction to the Aquatic Insects of North America, Kendall/Hunt Publishing Co., Dubuque, IA, 441 pp., 1978.

SNAILS AND LIMPETS
(PHYLUM MOLLUSCA, CLASS GASTROPODA)

Snails are commonly found in terrestrial, marine, and aquatic habitats. Most freshwater habitats, including small ponds, temporary pools, intermittent streams, brooks, and large lakes and rivers, have their characteristic population of snails. The vast majority of freshwater gastropods have a spiral or discoidal shell. A few genera (the freshwater limpets) have the shell in the form of a very low cone.

Gastropods require water high in dissolved salts, especially calcium carbonate, for shell construction. Thus they are found more often in alkaline, hard waters. Dissolved oxygen is another important limiting factor, rather high concentrations are usually required. For this reason grossly polluted rivers and lakes are usually devoid of non-pulmonate (non-air-breathing) gastropods.

Snails may be collected by dredge from a boat, a long-handled dip net, or handpicking. The soft body and shell of the snail must be preserved in formalin for accurate identification.

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HANDBOOK FOR EVALUATING ECOLOGICAL EFFECTS OF POLLUTION AT DARC--ETC(U)
MAY 80 C F PINKHAM, D A GAUTHIER
UNCLASSIFIED DPG-H8-80-805 NL

CLAMS AND MUSSELS
(PHYLUM MOLLUSCA, CLASS PELECYPODA)

The bivalve mollusks show a great variation in size. The small fingernail clams attain a length of only about 2 mm while some of the larger river clams are up to 250 mm long. They are generally sensitive to unfavorable environmental conditions such as heavy silt load, lack of food, low oxygen supply, and low water levels.

Pelecypods are found in most permanent bodies of water. Fingernail clams are fairly common in small creeks and tolerate water that is slightly acidic. Bivalves are most abundant in the shallows. The largest species and the greatest concentration of mussels and clams are found in large, unpolluted rivers and lakes. They prefer substrates composed of sand and gravel. Soft mud bottoms with accompanying high turbidity generally are uninhabited by bivalves.

Bivalves are excellent organisms to use for studies of biconcentration because they filter large quantities of water through their gills and store the contaminants and food in their fat. They also incorporate heavy metals and radionuclides in their shells. Each year's burden is deposited in the annual growth ring, so it is possible to investigate chronologically historical contamination within a drainage basin.

Clams and mussels may be collected in the same way as gastropods. Due to pollution, silting, and water projects, many of the less common species of bivalves are fast disappearing, resulting in their inclusion on state and federal endangered species lists.

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